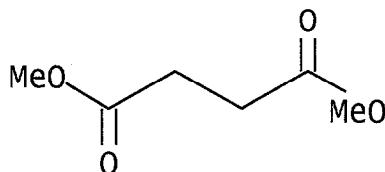


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**Robust Summaries for Dibasic Esters Solvents:
Dimethyl Succinate (DMS)****1. Substance Information****1.1. Chemical Name:** Dimethyl Succinate**1.2. CAS Registry No:** 106-65-0**1.3. Component CAS Nos.:** Not Applicable**1.4. Structural Formula:****1.5. Other Names:**

Dimethyl butanedioate, dibasic acid ester (dimethyl succinate), dimethyl ester, mixture with dimethyl butanedioate, dibasic dimethyl esters of succinic acid, and DMS.

2. Physical-Chemical Properties**2.1. Melting Point**

Value: 19 °C
Decomposition: No Data
Sublimation: No Data
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available.
Reference: IUCLID. (2000). IUCLID Dataset. European Chemicals Bureau, European Commission. Datasheet for dibasic esters, 2/18/00 [Subsequently referenced as IUCLID (2000)].

Additional References for Melting Point:

Dupont Co. (2001). Material Safety Datasheet DU000081 for DBE-4 (dimethyl succinate, DMS).

HSDB. (2001). Hazardous Substances Data Bank (HSDB/5370)

2.2. Boiling Point

Value: 196 °C
Decomposition: No Data
Pressure: 1013 hPa
Method: No Data
GLP: No Data

Reliability: Not assignable because limited study information was available
Reference: IUCLID (2000)

Additional References for Boiling Point:

Dupont Co. (2001). Material Safety Datasheet DU000081 for DBE-4 (dimethyl succinate, DMS).

Merck and Co. (1996). The Merck Index., Merck and Co., Inc., Whitehouse Station, NJ. (HSDB/5370)

2.3. Density

Value: 1.11 g/cm³
Temperature: 25°C
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available
Reference: IUCLID (2000)

Additional References for Density:

Dupont Co. (2001). Material Safety Datasheet DU000081 for DBE-4 (dimethyl succinate, DMS).

Merck and Co. (1996). The Merck Index., Merck and Co., Inc., Whitehouse Station, NJ. (HSDB/5370)

2.4. Vapor Pressure

Value: 0.03 hPa
Temperature: 20°C
Decomposition: No Data
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available
Reference: IUCLID (2000)

Additional References for Vapor Pressure:

Dupont Co. (2001). Material Safety Datasheet DU000081 for DBE-4 (dimethyl succinate, DMS).

2.5. Partition Coefficient (log K_{ow})

Value: 0.19 (measured)
Temperature: 25°C
Reliability: Not assignable because limited study information was available
Reference: IUCLID (2000)

Additional References for Partition Coefficient:

Dupont Co. (2001). Material Safety Datasheet DU000081 for DBE-4 (dimethyl succinate, DMS).

Hansch, C. et al. (1995). Exploring QSAR. Hydrophobic, Electronic and Steric Constants, p. 48, Amer. Chem. Soc., Washington, DC (HSDB/5370).

2.6. Water Solubility

Value: 131 g/L
Temperature: 25°C
pH/PKa pH 4-5
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available
Reference: IUCLID (2000)

Additional References for Water Solubility:

Dupont Co. (2001). Material Safety Datasheet DU000081 for DBE-4 (dimethyl succinate, DMS).

Merck and Co. (1996). The Merck Index., Merck and Co., Inc., Whitehouse Station, NJ. (HSDB/5370).

2.7. Flash Point

Value: 94 °C
Method: Tag Closed Cup
GLP: No
Reliability: Not assignable because limited study information was available
Reference: Dupont Co. (2001). Material Safety Data Sheet DU000150

Additional References for Flash Point:

IUCLID (2000)

3. Environmental Fate

3.1. Photodegradation

Type:	Photodegradation in Air
Indirect Photolysis:	No Data
Sensitizer:	OH
Concentration	1,500,000 molecules/cm ³
Rate constant:	4.32×10^{-13} cm ³ /molecules/sec
Degradation:	ca. 50% after 24.8 days
Method:	other (calculated)
GLP:	No
Reliability:	Not assignable because limited study information was available
Reference:	IUCLID (2000)

3.2. Stability in Water

Concentration:	No Data
Half-life:	No Data
Percent Hydrolyzed:	No Data
Method:	No Data
GLP:	No Data
Reference:	No Data
Reliability:	No Data

3.3. Transport (Fugacity)

Media:	Air, water, soil, and sediment	
Distributions:	Air	4.67%
	Water	48.2%
	Soil	47.1%
	Sediment	0.0818%
Adsorption		
Coefficient:	Not applicable	
Desorption:	Not applicable	
Volatility:	Not applicable	
Method:	Calculated method based on Mackay's Level III Fugacity model and using Estimations Programs Interface (EPIWIN v. 3.10)	

Data Used:

Molecular Weight: 146.14
Henry's Law Constant: 3.2×10^{-7} atm.-m³/mole
(calculated VP/Wsol)
Vapor Pressure: 0.218 mm Hg (user –entered)
Log Kow: 0.35 (KOWWIN Program).
Soil Koc: 0.918 (calculated by model)
GLP: No
Reliability: Estimated value based on accepted SAR model.
Reference: SRC and EPA developed models EPIWIN (v. 3.10) which contains a Level III Fugacity Model developed by Dr. Donald MacKay and Co-Workers which is detailed in:

Mackay, D. (1991). Multimedia Environmental Models: the Fugacity Approach. pp 67-183, Lewis Publishers, CRC Press.

MacKay, D. et al. (1996) Environ. Toxicol. Chem., 15(9):1618-1626.

MacKay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

3.4. Biodegradation

Type: Aerobic
Inoculum: activated sludge, industrial, non-adapted
Concentration: 600 mg/L related to COD (Chemical Oxygen Demand)
Degradation: > 95% after 3 days
Kinetics: 3 hour = 11%
Method: OECD 302 B “Inherent biodegradability: modified Zahn-Wellens Test”
GLP: Yes
Reliability: High (Scientifically defensible or guideline method, GLP)
Reference: IUCLID (2000)
Remarks: Dimethyl succinate was >95% degraded after three days and after 10 days in an aerobic test using activated sludge, industrial, non adapted. Adsorption at three hours was 11% and adsorption after one day was 69%.

Additional References for Biodegradation:

Based upon a group contribution method for predicting the probability and rate of aerobic biodegradation, dimethyl succinate has been estimated to be highly biodegraded with complete biodegradation occurring over a period of weeks.

Boethling, R.S., et al. (1994). Environ. Sci. Technol. 28:459-65 (HSDB/5370).

SRC (n.d.) Syracuse Research Corporation (HSDB/5370).

3.5. Bioconcentration

Value: 1.1 (calculated)
Method: Regression derived equation (Lyman et al.1990)
GLP: No Data
Reliability: Estimated value based on accepted SAR model.
Reference: HSDB/5370
Remarks: An estimated BCF value of 1.1 was calculated for dimethyl succinate (SRC, n.d.), using a measured log Kow of 0.35 (Hansch et al., 1995) and a recommended regression-derived equation (Lyman et al., 1990). According to a classification scheme (Franke et al., 1994), this BCF value suggests that bioconcentration in aquatic organisms is low (SRC, n.d.).

Additional References for Bioconcentration:

Franke, C. et al. (1994). Chemosphere, 29:1501-14 (HSDB/5021)
Hansch, C. et al. (1995). Exploring QSAR. Hydrophobic, Electronic and Steric Constants, p. 48, Amer. Chem. Soc., Washington, DC (HSDB/5021).
SRC (n.d.) Syracuse Research Corporation (HSDB/5021).

4. Ecotoxicity

4.1. Acute Toxicity to Fish

Type: **96-h LC₅₀**
Species: *Brachydanio rerio*
Value: 50 – 100 mg/L
Method: OECD 203
Year: 1990
GLP: No Data
Reference: IUCLID (2000)
Reliability: Not assignable because limited study information was available

Additional References for Bioconcentration:

None Found

4.2. Acute Toxicity to Invertebrates

Type: 48-h LC50
Species: Daphnia magna
Value: 3317.276 mg/L
Method: Calculated using ECOSAR (v. 0.99g) developed for the EPA by Syracuse Research Center (SRC) (© 2000 US EPA).
GLP: No
Reliability: Estimated value based on accepted SAR model.
Reference: Meylan, W.M. and P.H. Howard. (1999). User's Guide for the ECOSAR Class Program. Version 0.99e (Mar 1999), prepared for J. Vincent Nabholz and Gordon Cash, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, North Syracuse, NY 13212.

4.3. Acute Toxicity to Aquatic Plants

Type: 96-h EC50
Species: Green algae
Value: 11.917 mg/L
Method: Calculated using ECOSAR (v. 0.99g) developed for the EPA by Syracuse Research Center (SRC) US EPA).
GLP: No
Reliability: Estimated value based on accepted SAR model.
Reference: Meylan, W.M. and P.H. Howard. (1999). User's Guide for the ECOSAR Class Program. Version 0.99e (Mar 1999), prepared for J. Vincent Nabholz and Gordon Cash, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, North Syracuse, NY 13212.

5. Mammalian Toxicity

5.1. Acute Toxicity

Type: Oral LD50
Guideline: EPA 40 CFR 798.1175
Species/Strain: Sprague Dawley Rats
Value: > 500 mg/kg b. wt. and < 5000 mg/kg b.wt.
Method: DMS was administered a single dose via oral gavage at two dose levels (500 and 5000 mg/kg b.wt.) with a 14 day observation period. Test material was administered as

	received. 5 females and 5 males were used for each dose level. Necropsies were performed on animals that died and that survived to 14 days post-administration.
GLP:	Yes
Test Substance:	99.5% DMS
Results:	At 500 and 5,000 mg/kg b.wt. mortality rates were 0/10 and 10/10, respectively. The mortalities occurred in the first day (1, 2, 4, and 6 hours). In the 500 mg/kg treatment level no abnormalities were found throughout the study. In the 5,000 mg/kg treatment group observations included: Antemortem lethargy in most animals and moist rales in one female. Surviving animals from the 500 mg/kg treatment group showed no macroscopic abnormalities with the exception of two animals that exhibited changes in the kidney (dilated renal pelvis, discoloration, tan nodules) and pale white areas on the spleen (one animal). Necropsies of dead animals revealed discoloration/edema in the lungs. Other observations in single animals were enlarged and reddened liver, discoloration of kidney or stomach, and kidney and urinary tract changes.
Reference:	Bio/dynamics Inc. (1992). Acute Oral Toxicity in Rats (DMS, Dimethyl Succinate). Submitted to Monsanto Company Reference Numbers: 92-6320, BD-92-245, October 22, 1992.
Reliability:	High (Scientifically defensible, or guideline method, GLP)
Remarks:	This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Oral Toxicity:

Rat LD50 = 6892 mg/kg b.wt. IUCLID (2000)
Rat LD50 > 5000 mg/kg b.wt. IUCLID (2000)

Type:	Dermal LD50
Guideline:	EPA 40 CFR 798.1100
Species/Strain	New Zealand White Rabbits
Value:	> 5000 mg/kg b.wt.
Method:	DMS was administered a single dose applied directly to the skin over a 12 x 14 cm area approximating 10% of the body surface. Contact with excess material was maintained for 24 hours and animals were observed for 14 days after

initiation of dose. A total of 5 females and 5 males were used for this study. Test material was administered as received.

GLP: Yes
Test Substance: 99.5% DMS
Results: All animals survived after dermal treatment at 5,000 mg/kg b. wt. Animals gained weight or remained stable during 14 days post-treatment, with the exception of two animals which showed slight losses of weight during one of the two 7-day periods. All animals were free of systemic toxicity throughout the study and no abnormalities were observed during post-mortem macroscopic observation. However, one animal exhibited decreased fecal volume and decreased food consumption on days 3 and 4 (collar caught in mouth). Macroscopic examinations revealed no observable abnormalities with the exception of fibrous white material found in the stomach of one male.
Reference: Bio/dynamics Inc. (1992). Acute Dermal Toxicity in Rabbits (DMS, Dimethyl Succinate). Submitted to Monsanto Company Reference Numbers: 92-6321, BD-92-245, October 22, 1992.
Reliability: High (Scientifically defensible, or guideline method, GLP)
Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Dermal Toxicity:

Rabbit Dermal LD₅₀ > 5000 mg/kg b.wt. IUCLID (2000)

Type: Acute Inhalation
Value: No Data found

Type: Primary Dermal Irritation
Guideline: EPA 40 CFR 798.4470
Species/Strain: New Zealand White Rabbits
Sex/Number: Six (4 males and 2 females)
Value: Average Dermal Irritation Score (ADIS) was 0.0
Method: DMS was administered to six animals (3 female/3 male) as a single dose applied directly to two 1 x 1 inch areas of skin on the back and held in place with semi-occlusive dressings for 4 hours. Animals were observed for subsequent 3 days and treated areas were observed at 30 minutes and 24, 48, and 72 hours. Test material was administered as received.
GLP: Yes

Test Substance: 99.5% DMS

Results: The ADIS for DMS is 0.0. No irritation was observed throughout the study. This material would probably not be considered to produce dermal irritation as defined in EPA Guidelines.

Reference: Bio/dynamics Inc. (1992). Primary Dermal Irritation Study in Rabbits (DMS, Dimethyl Succinate). Submitted to Monsanto Company Reference Numbers: 92-6322, BD-92-245, October 22, 1992.

Reliability: High (Scientifically defensible, or guideline, GLP)

Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Dermal Irritation:

Dupont Co. (1975). Unpublished data, Haskell Laboratory Report No.154-75.

Dupont Co. (1975). Unpublished data, Haskell Laboratory Report No.299-75.

IUCLID (2000)

Type: Eye Irritation

Guideline: EPA 40 CFR 798.4500

Species/Strain: New Zealand White Rabbits

Value: DMS produced mild to moderate, transient ocular irritation.

Method: A single ocular administration of DMS (0.1 ml) was applied to 2 male and 4 female adults rabbits followed by observations at 1, 24, 48, 72 hours and 7 and 10 days. The observation period continued up to 10 days or until no signs of irritation were present. The cornea, iris and conjunctivae were observed and lesions were graded. Test material was administered as received.

GLP: Yes

Test Substance: 99.5% DMS

Results: DMS produced mild to moderate, transient ocular irritation. This material would be considered to produce eye irritation as defined in the EPA Guidelines. All six animals exhibited slight to moderate irritation of the conjunctivae (redness, chemosis, and discharge). Four animals exhibited irridial and corneal changes. Corneal changes included slight dulling or opacity of the corneal surface and corneal ulceration (one animal exhibited corneal stippling). One of six animals was free of all ocular irritation by 24 hours post-administration and three by 48 and 72 hours with the remaining two animals free of irritation by day 7.

Reliability: High (Scientifically defensible, or guideline method, GLP)

Reference: Bio/dynamics Inc. (1992). Primary Eye Irritation Study in Rabbits (DMS, Dimethyl Succinate). Submitted to Monsanto Company Reference Numbers: 92-6319, BD-92-242, October 22, 1992.

Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Eye Irritation:

Dupont Co. (1975). Unpublished data, Haskell Laboratory Report No.302-75.

IUCLID (2000)

5.2. Repeated Dose Toxicity:

Type: 90-day Inhalation

Guideline: Based on 40CFR799.9346, 799.9380, 799.9620, plus cell proliferation study.

Species/Strain: Rats/ CrI:CD®(SD)IGS BR

Sex/Number: 36 males/36 females per treatment level

Route of Administration: inhalation

Exposure Period: 90 days

Frequency Of treatment: 6 hours/day, 5 days/wk

Exposure levels: 0 or 400 mg/m³ DMS

Methods: Groups of male and female (nulliparous and non-pregnant) rats were exposed via inhalation to DMS over a 90-day period. The exposure period was followed by a 1-month recovery period. Rats were weighed once per week and clinical signs were taken daily. Food consumption was determined on a weekly basis. Samples for hepatic, lung, and nasal (levels II and III) cell proliferation (CP) were collected from rats approximately 2 weeks after initiation of the study and approximately 90 days after study initiation. A clinical pathology evaluation was conducted on rats approximately 45 and 90 days after initiation of the study. Approximately 90 days after study initiation, rats designated for the clinical pathology evaluation were sacrificed for pathological examination and evaluation of male reproductive endpoints, including sperm motility, sperm number, and sperm morphology. A neurobehavioral test battery, consisting of functional observational battery assessments and motor activity, was conducted prior to test

substance administration to obtain baseline measurements, and during test weeks 4, 8, 13, and 18 (recovery). Rats designated for neuropathological evaluation were sacrificed approximately 90 days after study initiation and after approximately 1 month of recovery. The estrous cycle of female rats was determined for the last 21 days of exposure. Following 90 days of exposure, blood was collected via the tail vein from male and female rats and serum was subjected to hormonal analyses. In male rats, serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone concentrations were measured. In female rats, serum estradiol and progesterone concentrations were measured.

GLP:

Yes

Test Substance:

99.198% DMS

Results:

The analytically determined overall mean concentration of DMS in the exposure chambers targeted to 400 mg/m³ were 400 mg/m³. The overall mean temperature in each of the exposure chambers ranged from 21-22°C. The overall mean relative humidity in each of the exposure chambers ranged from 35-55%, and the oxygen concentration was approximately 21 %. The mean chamber airflows ranged from 320-330 L/min in the 1.4-m³ control chambers and 1600-1800 L/min in the 9-m³ test chambers.

No concentration-related effects were observed on mortality, clinical signs of toxicity, body weights, body weight gains, food consumption, food efficiency, clinical pathology, neurobehavioral endpoints, neuropathology, sperm motility or morphology, or estrous cycle.

Compound-related effects were observed in the noses of male and female rats exposed to 400 mg/m³ DMS for 90 days. These effects consisted primarily of degeneration/atrophy of the olfactory mucosa of the dorsal meatus and of the dorsomedial aspect of the dorsal endoturbinate. Less commonly, focal respiratory metaplasia of the olfactory mucosa of the dorsal meatus was also present. Lesions were minimal to mild in severity.

Degeneration/atrophy of the olfactory mucosa occurred in recovery animals in the same locations as was apparent at the 90-day sacrifice. The lesions were usually focal and minimal in severity. Male rats exposed to 400 mg/m³ DMS showed significant increased CP in the liver at day 14 compared to controls. Female rats exposed to 400 mg/m³ DMS had significantly greater CP in the nose level III relative to controls at day 90. In female rats, DMS caused a

	statistically significant decrease in serum estradiol concentrations (43% of control); serum progesterone concentrations were not affected. In male rats, epididymal sperm counts (per cauda and per gram cauda epididymis) were significantly increased (153 and 141% of control, respectively).
Reliability:	High (Scientifically defensible, or guideline method, GLP). This study is part of a much larger study that included DMA and DMG as well with dose response information for DMG.
Reference:	Dupont Co. (2000). Unpublished data, Haskell Laboratory: MR-13128-1, Dupont-3557.
Remarks:	The NOEL for this study is defined as the highest dose at which toxicologically important effects attributable to the test substance were not detected. Thus, for this study, the NOEL is equivalent to the NOEL as defined by the United States Environmental Protection Agency (1985) and to the no-observed-adverse-effect level (NOAEL) as defined by the European Union (1994).

5.3. Developmental Toxicity

Value: No Data Found

5.4. Reproductive Toxicity

Type:	90-day
Species/Strain:	Rats/ Crl:CD®(SD)IGS BR
Sex/Number:	
Route of Administration:	inhalation
Exposure Period:	90 days
Frequency Of treatment:	6 hours/day, 5 days/wk
Exposure levels:	0 or 400 mg/m ³ DMS
Methods:	Male and female rats were exposed via inhalation to 0 or 400 mg/m ³ dimethyl succinate (DMS) over a 90-day period. The exposure period was followed by a one-month recovery period. Approximately 90 days after study initiation, rats in the clinical pathology subgroups were sacrificed and evaluated for sperm motility, sperm number, and sperm morphology. The estrous cycle of female rats was determined for the last 21 days of exposure. Hormonal analyses were conducted following 90 days of exposure.

Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone concentrations were measured in the male rats and serum estradiol and progesterone concentrations were measured in the female rats.

GLP: Yes
 Test Substance: 99.198% DMS
 Results: No concentration-related effects were observed on sperm motility or morphology, or estrous cycle. In female rats, DMS caused a statistically significant decrease in serum estradiol concentrations (43% of control); serum progesterone concentrations were not affected. In male rats, epididymal sperm counts (per cauda and per gram cauda epididymis) were significantly increased (153 and 141% of control, respectively). Additional details of this study can be found in the sub chronic inhalation section (DuPont Co., 2000).
 Reliability: High (Scientifically defensible, or guideline method, GLP)
 Reference: Dupont Co. (2000). Unpublished data, Haskell Laboratory: MR-13128-1, Dupont-3557.

5.5. Genetic Toxicity *in vitro* (gene mutations)

Type: Ames Test
 Tester strains: TA 98, TA 100, TA 1535, and TA 1537
 Exogenous
 Metabolic
 Activation: With and without
 Exposure
 Concentrations: 32, 160, 800, 20000 µg/Platte
 Methods: No Data
 GLP: No
 Test material: DMS
 Results: Negative
 Reliability: Not assignable because limited study information was available
 Reference: IUCLID (2000)
 Remarks: All references found reported DMS to be negative for mutagenicity.

Additional References for *in vitro* Genetic Toxicity:

Andersen, P.H. and N.J. Jensen (1984). Food Additive Contam., 1(3):283-288 (J-7801).
 Zeiger, E. et al. (1992). Environ. Mol. Mutagen., 19(Suppl. 21):2-141.

5.6. Genetic Toxicity *in vivo* (chromosomal aberrations)

Type:	<i>In vivo</i> Rat Micronucleus Test
Methods:	No Data
Results:	Dimethyl succinate (DMS) was negative in an <i>in vivo</i> micronucleus test using Fischer rats.
Reference:	NTP. (1997). National Toxicology Program, Unpublished results (C-5143).

6.0 Other Information

6.1 Biochemical/Metabolism Studies

Hepatic mitochondria were used as a model for nasal tissue. DBEs were found to inhibit mitochondrial ATP synthesis 11 to 27% at 100 μ M. The order of potency was DMA > DMG > DMS and paralleled the V_{\max}/K_m values for the hydrolysis of the DBEs to their monomethyl esters. Pre-treatment of the rats with 100 mg/kg of bis-nitrophenyl phosphate for three days decreased the rate of hydrolysis of the DBEs approximately 50% and protected the mitochondria from DBE-induced inhibition of ATP synthesis. These results support the hypothesis that DBE-induced cytotoxicity results from esterase-mediated hydrolysis to acid metabolites and interference with intermediary metabolism (Bogdanffy and Londergan, 1989).

In the study cited above, DBE cytotoxicity was shown to be due to esterase-mediated activation. In this present study, the putative toxic monomethyl and diacid metabolites were evaluated in an *in vitro* nasal explant system. Monomethyl adipate (MMA), glutarate (MMG), and succinate (MMS) induced increases in nasal explant acid phosphatase release, a biochemical index of their cytotoxicity. Metabolism of MMA and MMG to their diacids paralleled cytotoxicity. MMS metabolism was not quantifiable. Pretreatment of rats with a carboxylesterase inhibitor reduced cytotoxicity and metabolism of MMA and MMG, but not cytotoxicity of MMS. It is concluded that both monomethyl ester and diacid metabolites of DBE are cytotoxic. The contribution of each to cytotoxicity *in vivo* may depend on their rate of formation during exposure (Bogdanffy et al., 1991a; Trela and Bogdanffy, 1991b).

The kinetic parameters V_{\max} , K_m , K_{si} , and V/K were measured for the hydrolysis of the dibasic esters in the target nasal tissue, olfactory mucosa, and non-target tissue, respiratory mucosa. It was determined under the conditions of these experiments, diacid metabolites were not formed. Esterase activity was inhibited by pretreatment with bis-nitrophenyl phosphate. V_{\max} values for the three dibasic esters were 5- to 13-fold greater in olfactory mucosa than respiratory mucosa for male and female

rats. V/K values were 4- to 11-fold greater in olfactory mucosa than respiratory mucosa for male and female rats. V/K was similar between male and female olfactory mucosa when DMG was used as the substrate. With DMS or DMA as the substrate, V/K for female olfactory tissue was 0.5- or 2-fold that of males, respectively. Differences in V/K were mainly due to decreases in K_m associated with increasing carbon chain length. Substrate inhibition was observed at DBE concentrations greater than approximately 25 mM, which are unlikely to be achieved *in vivo*. These results lend further support to the hypothesis that organic acid accumulation in the target tissue, olfactory mucosa, plays a significant role in the pathogenesis of DBE-induced nasal lesions (Bogdanffy et al., 1991b).

Since female rats appear to be more sensitive to DBE-induced olfactory toxicity than males, it was of interest to measure the rate of hydrolysis of DBEs in male and female nasal mucosa homogenates and compare these values to those derived from human nasal tissue obtained at autopsy. For both male and female rats, V_{max}/K_m values followed the order DMA > DMG > DMS paralleling carbon chain length. The V_{max}/K_m values for female olfactory mucosa using DMA or DMS as substrates were two times or one-half the values for male olfactory mucosa, respectively. Hydrolysis of DBEs was detectable in only three of six human samples. Activity values that were measurable were two or three orders of magnitude lower than that of rat respiratory or olfactory mucosa, respectively. These data suggest the rate of conversion of DBEs to acid metabolites in nasal tissue is less significant in humans than in rats, and that the rat may be more sensitive than man to the effects of DBEs on nasal mucosa (Kee et al., 1989).

The enzymatic esterase activity of carboxylesterases is integral to the nasal toxicity of many esters, including DMG, DMS, and DMA. Inhalation of these esters specifically damages the olfactory mucosa of rodents. In this study, the localization differential distribution of a 59 KD carboxylesterase was demonstrated in the nasal tissues of the rat by immunohistochemistry. Rabbit antiserum against the 59 KD rat liver microsomal carboxylesterase bound most prominently to the olfactory mucosa when applied to decalcified, paraffin-embedded sections of rat nasal turbinates. Within the olfactory mucosa, anti-carboxylesterase did not bind to sensory neurons, the target cell for ester-initiated toxicity; these cells apparently lack carboxylesterase. Instead, the antibody was preferentially bound by cells of Bowman's glands and sustentacular epithelial cells that are immediately adjacent to the olfactory nerve cells. In contrast, non-olfactory tissues (respiratory mucosa and squamous epithelium) which are more resistant to the toxicity of esters, had less carboxylesterase content (Olson et al., 1993).

An *in vitro* system was utilized to determine if DBE toxicity is dependent on metabolic activation by carboxylesterase. Explants from the olfactory

and respiratory regions of the rat nasal cavity were incubated in a medium containing 10-100 mM of the dimethyl esters of adipic-, glutaric-, and succinic acids. DBE caused a dose-related increase in nasal explant acid phosphatase release, a biochemical index of cytotoxicity. A parallel increase in carboxylesterase-mediated monomethyl ester (MME) formation was seen. In addition, MME concentrations and acid phosphatase release were generally higher in olfactory than respiratory tissues. DME-induced cytotoxicity and MME formation were markedly reduced in nasal tissue excised from rats treated with a carboxylesterase inhibitor, bisnitrophenyl phosphate (Trela and Bogdanffy, 1990; 1991a).

The kinetic constants were determined for carboxylesterase-mediated hydrolysis of DBEs and correlated with lesion formation. No diacid metabolites were found. V max values for the formation of MMS, MMG, and MMA were approximately 8- to 10-times larger in olfactory mucosa than in respiratory mucosa. V/K values for the formation of MMG and MMA were approximately 9- and 10-times larger in olfactory mucosa than respiratory mucosa. For the formation of MMS, V/K was approximately 2 times larger in respiratory mucosa than olfactory mucosa (Patterson et al., 1988).

To determine the biochemical mechanism for the toxic effect of DBE on rat nasal olfactory mucosa, an *in vitro* study was conducted with rat and human nasal tissue. This study demonstrated that the nasal tissue toxicity of DBE is related to enzymatic hydrolysis of DBE within the nasal cavity to form the corresponding monomethyl ester. Additionally, it was found that human nasal tissue hydrolyzes DBE at 1/100 to 1/1000 the rate of rat nasal tissue. For this reason, the nasal tissue of humans is likely to be at greatly reduced risk of DBE toxicity compared to rats (Bogdanffy and Frame, 1994).

- References:
- Bogdanffy, M. S. and T. Londergan (1989). The Toxicologist, 9(1):249 (Abstract 996).
 - Bogdanffy, M. S. et al. (1991a). The Toxicologist, 11(1):182 (Abstract 664).
 - Bogdanffy, M. S. et al. (1991b). Drug Metab. Dispos. Biol. Fate Chem., 19(1):124-129.
 - Bogdanffy, M. S. and S. R. Frame (1994). Inhalation Toxicology, 6(Supplement):205-: (NIOSH/00224297) (as cited in Dupont Co. (1995). AEL Documentation, October 4..
 - Kee, C. R. et al. (1989). The Toxicologist, 9(1):284 (Abstract 1139).

Olson, M. J. et al. (1993). J. Histochem. Cytochem., 41(2):307-311(BIOSIS/93/13203).

Patterson, C. A. et al. (1988). The Toxicologist, 8(1):6 (Abstract 22).

Trela, B. A. and M. S. Bogdanffy (1990). The Toxicologist, 10(1):261 (Abstract 1044).

6.

Robust Summaries for Dibasic Ester Solvents: Dimethyl Glutarate (DMG)

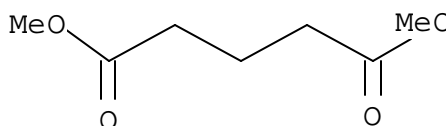
1. Substance Information

1.1. Chemical Name: Dimethyl Glutarate

1.2. CAS Registry No: 1119-40-0

1.3. Component CAS Nos.: Not Applicable

1.4. Structural Formula:



1.5. Other Names: Dibasic acid ester (dimethyl glutarate), DBE-5, dimethyl pentanedioate, dibasic dimethyl esters of glutaric acid, methyl glutarate, and DMG.

2. Physical-Chemical Properties

2.1. Melting Point

Value:	-37°C
Decomposition:	No Data
Sublimation:	No Data
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	Dupont Co. (2001). Material Safety Data Sheet DU000150

Additional References for Melting Point:

IUCLID (2000). IUCLID Dataset. European Chemicals Bureau, European Commission. Datasheet for dimethyl glutarate, 2/18/00 [Subsequently referenced as IUCLID (2000)]

HSDB (2001). Hazardous Substances Data Bank (HSDB/5789)

The Merck Index (1976). 9th ed., p. 579, Merck & Co., Inc., Rahway, NJ (HSDB/5789).

Weast, R. C. (1979). CRC Handbook of Chemistry and Physics, 60th ed., p. C-415, CRC Press, Inc., Boca Raton, FL. (HSDB/5789).

2.2. Boiling Point

Value:	213.5-214°C
Decomposition:	No Data
Pressure:	752 mm Hg
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	Dupont Co. (2001). Material Safety Data Sheet DU000150

Additional References for Boiling Point:

IUCLID (2000)

The Merck Index (1976). 9th ed., p. 579, Merck & Co., Inc., Rahway, NJ (HSDB/5789).

2.3. Density

Value:	1.0876 g/cm ³
Temperature:	20°C
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	Dupont Co. (2001). Material Safety Data Sheet DU000150

Additional References for Density:

IUCLID (2000)

Weast, R. C. (1979). CRC Handbook of Chemistry and Physics, 60th ed., p. C-415, CRC Press, Inc., Boca Raton, FL. (HSDB/5789).

2.4. Vapor Pressure

Value:	0.7 mm Hg
Temperature:	20°C
Decomposition:	No Data
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	Dupont Co. (2001). Material Safety Data Sheet DU000150

Additional References for Vapor Pressure:

IUCLID (2000)

2.5. Partition Coefficient (log K_{ow})

Value: Log Kow 0.62 (calculated)
Method: Calculated using KOWWIN model (v. 1.66) in EPIWIN (v. 3.10) using experimental procedures of Hansch and Hoekman (1995).
GLP: No
Reliability: Estimated value based on accepted SAR model.
Reference: KOWWIN model (v. 1.66) in EPI (v. 3.10) Prepared by Syracuse Research Center (SRC) for J. Vincent Nabholz and Gordon Cash, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, North Syracuse, NY 13212 (© 2000 US EPA).

Hansch, C., A. Leo, D. Hoekman (1995). Exploring QSAR-Hydrophobic, Electronic, and Steric Constants. Washington, D.C., American Chemical Society

2.6. Water Solubility

Value: 4.3 wt%
Temperature: 20°C
pH/Pka: No Data
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available
Reference: Dupont Co. (2001). Material Safety Data Sheet DU000150

Additional References for Water Solubility:

IUCLID (2000)

Weast, R. C. (1979). CRC Handbook of Chemistry and Physics, 60th ed., p. C-415, CRC Press, Inc., Boca Raton, FL. (HSDB/5789).

2.7. Flashpoint

Value: 100°C
Method: Tag Closed Cup
GLP: No
Reliability: Not assignable because limited study information was available
Reference: Dupont Co. (2001). Material Safety Data Sheet DU000150

3. Environmental Fate

3.1. Photodegradation

Concentration:	No Data
Temperature:	No Data
Direct Photolysis:	No Data
Indirect Photolysis:	No Data
Breakdown	
Products:	No Data
Method:	No Data
GLP:	No Data
Reliability:	No Data
Reference:	No Data

3.2. Stability in Water

Concentration:	No Data
Half-life:	No Data
Percent Hydrolyzed:	No Data
Method:	No Data
GLP:	No Data
Reliability:	No Data
Reference:	No Data

3.3. Transport (Fugacity)

Media:	Air, water, soil, and sediment	
Distributions:	Air	6.25%
	Water	51.1%
	Soil	42.6%
	Sediment	0.0882%
Adsorption		
Coefficient:	Not applicable	
Desorption:	Not applicable	
Volatility:	Not applicable	
Method:	Calculated method based on Mackay's Level III Fugacity model and using Estimations Programs Interface (EPIWIN v. 3.10)	
Data Used:		
	Molecular Weight:	160.17
	Henry's Law Constant:	3.43×10^{-6} atm.-m ³ /mole (calculated VP/Wsol)
	Vapor Pressure:	0.7 mm Hg (user –entered)
	Log Kow:	0.62 (KOWWIN Program).

	Soil Koc:	1.71 (calculated by model)
GLP:	No	
Reliability:	Estimated value based on accepted SAR model.	
Reference:	SRC and EPA developed models EPIWIN (v. 3.10) which contains a Level III Fugacity Model developed by Dr. Donald MacKay and Co-Workers which is detailed in:	

Mackay, D. (1991). Multimedia Environmental Models: the Fugacity Approach. pp 67-183, Lewis Publishers, CRC Press.

MacKay, D. et al. (1996) Environ. Toxicol. Chem., 15(9):1618-1626.

MacKay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

3.4. Biodegradation

Type:	Ready Biodegradability
Guideline:	OECD Guideline 301 C - Ready Biodegradability: Modified MITI Test (I).
Method:	DMG was 98% degraded after 28 days in an aerobic test using activated sludge, non-adapted, as the inoculum. A concentration of 100 mg/L related to the test substance was used. DMG was readily biodegradable in this test. The BOD/COD ratios for 5, 10, 15, 20, and 28 days were 44, 55, 64, 69, and 75%.
GLP:	No
Reliability:	Not assignable because limited study information was available
Reference:	IUCLID 2000)

Additional References for Biodegradation:

Rhone Poulenc (1990). Unpublished data (cited IUCLID 2000).

3.5. Bioconcentration

Value:	BCF of 3.162 (Log BCF of 0.5) (calculated)
Method:	Calculated using BCF Model (v. 2.14) in EPIWIN (v. 3.10)
GLP:	No
Reliability:	Estimated value based on accepted SAR model.
Reference:	EPIWIN (v. 3.10) Prepared by Syracuse Research Center (SRC) for J. Vincent Nabholz and Gordon Cash, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by

4. Ecotoxicity

4.1. Acute Toxicity to Fish

Type: 96-h LC₅₀
Species: *Lepomis macrochirus* (Bluegill Sunfish)
Value: 30.9 mg/L
Method: A static method with all glass chambers was used to treat 10 bluegill per chamber at nominal concentrations (See Table 4.1(a) below). Temperature was maintained at 20°C (± 0.2) and pH and dissolved oxygen were monitored. Nine exposure concentrations (range from 20 to 50 mg/L, nominal), a control, and a solvent control were used. The LC₅₀ was estimated using Finney (1952).

Nominal Conc. (mg/L)	Observed Mortality %	pH		Dissolved Oxygen	
		Initial	Final	Initial	Final
50	100	7.3	7.0	8.2	6.5
40	100	7.2	6.5	8.1	1.2
37.5	100	7.3	6.4	8.1	1.5
35	90	7.1	6.6	8.2	0.8
32.5	40	7.3	6.4	8.2	1.3
30	80	7.2	6.6	8.2	0.6
27.5	10	7.3	6.5	8.2	1.3
25	10	7.3	6.7	8.2	1.5
20	20	7.2	6.7	8.1	1.5
control	0	7.4	6.8	8.2	3.8
Acetone control	0	7.0	6.7	8.1	1.7

Test Substance: 98% DMG
GLP: No
Reliability: Moderate (Scientifically defensible, non-GLP)
Reference: Dupont Co. (1976). Unpublished data, Haskell Laboratory Report No. 679-76.

4.2. Acute Toxicity to Invertebrates

Type: 48-h LC₅₀
Species: Daphnia magna

Value:	1,275 mg/L
Method:	Calculated using ECOSAR (v. 0.99g) developed for the EPA by Syracuse Research Center (SRC) (© 2000 US EPA).
GLP:	No
Reliability:	Estimated value based on accepted SAR model.
Reference:	Meylan, W.M. and P.H. Howard. (1999). User's Guide for the ECOSAR Class Program. Version 0.99e (Mar 1999), prepared for J. Vincent Nabholz and Gordon Cash, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, North Syracuse, NY 13212.

4.3. Acute Toxicity to Aquatic Plants

Type:	96-h EC50
Species:	Green algae
Value:	7.186 mg/L
Method:	Calculated using ECOSAR (v. 0.99g) developed for the EPA by Syracuse Research Center (SRC) US EPA).
GLP:	No
Reliability:	Estimated value based on accepted SAR model.
Reference:	Meylan, W.M. and P.H. Howard. (1999). User's Guide for the ECOSAR Class Program. Version 0.99e (Mar 1999), prepared for J. Vincent Nabholz and Gordon Cash, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, North Syracuse, NY 13212.

5. Mammalian Toxicity

5.1. Acute Toxicity

Type:	Oral LD50
Guideline:	EPA (40 CFR) 798.1175
Species/Strain	Sprague Dawley Rats
Value:	> 5000 mg/kg b. wt.
Method:	DMG was administered a single dose via oral gavage at 5000 mg/kg b.wt. with a 14 day observation period. Test material was administered as received. 5 females and 5 males were used for each dose level. Necropsies were performed on animals that died and that survived to 14 days post-administration.

GLP:	Y
Test Substance:	99.5% DMG
Results:	At 5,000 mg/kg b.wt. all animals survived to 14 days. Two animals exhibited moistened rales on the day of application and otherwise, surviving animals showed no abnormalities. All animals killed on day 14 were free of abnormalities with the exception of two animals that exhibited changes in the lungs (discoloration) or kidneys (dilated renal pelvis).
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Bio/dynamics Inc. (1992). Acute Oral Toxicity in Rats (DMG, Dimethyl Gluturate). Submitted to Monsanto Company Reference Numbers: 92-6324, BD-92-244, September 30, 1992.
Remarks:	This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Oral Toxicity:

Dupont Co. (1975a). Unpublished data, Haskell Laboratory Report No. 33-75.

Type:	Dermal LD50
Guideline:	EPA (40 CFR) 798.1100
Species/Strain	New Zealand White Rabbits
Value:	> 5000 mg/kg b. wt.
Method:	DMG was administered a single dose applied directly to the skin over a 12 x 14 cm area approximating 10% of the body surface. Contact with excess material was maintained for 24 hours and animals were observed for 14 days after initiation of dose. A total of 5 females and 5 males were used for this study. Test material was administered as received.
GLP:	Y
Test Substance:	99.5% DMG
Results:	All animals survived after dermal treatment at 5,000 mg/kg b. wt. Animals gained weight during 7 days post-treatment, but all animals lost weight or remained stable from day 7 to 14 post-treatment. All animals were free of systemic toxicity throughout the study, but three animals exhibited moderate lacrimation and clear nasal discharge. No abnormalities were observed during post-mortem macroscopic observation with the exception of a missing kidney in one animal and dilated ventricles of the brain filled with clear fluid in one animal.
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Bio/dynamics Inc. (1992). Acute Dermal Toxicity in Rabbits (DMG, Dimethyl Gluturate). Submitted to

Monsanto Company Reference Numbers: 92-6325, BD-92-244, September 30, 1992.

Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Dermal Toxicity:

Dupont Co. (1975). Unpublished data, Haskell Laboratory Report No. 155-75.

Dupont Co. (1975). Unpublished data, Haskell Laboratory Report No. 300-75.

Type:	Primary Dermal Irritation
Guideline:	EPA (40 CFR) 798.4470
Species/Strain	New Zealand White Rabbits
Value:	Average Dermal Irritation Score (ADIS) was 0.0
Method:	DME was administered to six animals (3 female/3 male) as a single dose applied directly to two 1 x 1 inch areas of skin on the back and held in place with semi-occlusive dressings for 4 hours. Animals were observed for subsequent 3 days and treated areas were observed at 30 minutes and 24, 48, and 72 hours. Test material was administered as received.
GLP:	Yes
Test Substance:	99.5% DMG
Results:	The ADIS for DMG is 0.0. No irritation was observed throughout the study. This material would not be considered to produce dermal irritation as defined in EPA Guidelines.
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Bio/dynamics Inc. (1992). Primary Dermal Irritation Study in Rabbits (DMG, Dimethyl Gluterate). Submitted to Monsanto Company Reference Numbers: 92-6326, BD-92-244, September 30, 1992.
Remarks:	This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Dermal Irritation: None found

Type:	Eye Irritation
Guideline:	EPA (40 CFR) 798.4500
Species/Strain:	New Zealand White Rabbits
Value:	DMG produced mild to moderate, transient ocular irritation.

Method:	A single ocular administration of DMG (0.1 ml) was applied to 3 male and 3 female adults rabbits followed by observations at 1, 24, 48, 72 hours and 7 and 10 days. The observation period continued up to 10 days or until no signs of irritation were present. The cornea, iris and conjunctivae were observed and lesions were graded. Test material was administered as received.
GLP:	Y
Test Substance:	99.5% DMG
Results:	DMG produced mild to moderate, transient ocular irritation. This material would be considered to produce eye irritation as defined in the EPA Guidelines. All six animals exhibited slight to moderate irritation of the conjunctivae (redness, chemosis, and discharge) and three exhibited corneal opacity or slight dulling of the corneal surface, four had corneal ulceration, and two had iridial changes. Four of six animals were free of all ocular irritation by 48 or 72 hours, with the remaining two animals free of irritation by Day 7.
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Bio/dynamics Inc. (1992). Primary Eye Irritation Study in Rabbits (DMG, Dimethyl Gluterate). Submitted to Monsanto Company Reference Numbers: 92-6327, BD-92-244, September 30, 1992.
Remarks:	This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Eye Irritation:

Dupont Co. (1975). Unpublished data, Haskell Laboratory Report No. 302-75.

5.2. Repeated Dose:

Type:	90-day Inhalation
Guideline:	Based on 40CFR799.9346, 799.9380, 799.9620, plus cell proliferation study.
Species/Strain:	Rats/Crl:CD®(SD)IGS BR
Sex/Number:	36 male/36 female per treatment level
Route of Administration:	Inhalation
Exposure Period:	90-days
Frequency of Treatment:	6 hours/day, 5 days/wk
Exposure Levels:	0, 10, 50, or 400 mg/m ³ dimethyl glutarate (DMG)

Method:	<p>Groups of male and female (nulliparous and non-pregnant) rats were exposed via inhalation to 0, 10, 50, or 400 mg/m³ DMG over a 90-day period. The exposure period was followed by a 1-month recovery period. Rats were weighed once per week and clinical signs were taken daily. Food consumption was determined on a weekly basis. Samples for hepatic, lung, and nasal (levels II and III) cell proliferation (CP) were collected from rats approximately 2 weeks after initiation of the study and approximately 90 days after study initiation. A clinical pathology evaluation was conducted on rats approximately 45 and 90 days after initiation of the study. Approximately 90 days after study initiation, rats designated for the clinical pathology evaluation were sacrificed for pathological examination and evaluation of male reproductive endpoints, including sperm motility, sperm number, and sperm morphology. A neurobehavioral test battery, consisting of functional observational battery assessments and motor activity, was conducted prior to test substance administration to obtain baseline measurements, and during test weeks 4, 8, 13, and 18 (recovery). Rats designated for neuropathological evaluation were sacrificed approximately 90 days after study initiation and after approximately 1 month of recovery. The estrous cycle of female rats was determined for the last 21 days of exposure. Following 90 days of exposure, blood was collected via the tail vein from male and female rats and serum was subjected to hormonal analyses. In male rats, serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone concentrations were measured. In female rats, serum estradiol and progesterone concentrations were measured.</p>
GLP:	Yes
Test Substance:	99.61% DMG
Results:	<p>The analytically determined overall mean concentrations of DMG in the exposure chambers targeted to 10, 50 or 400 mg/m³ were 10, 49 and 410 mg/m³, respectively. The overall mean temperature in each of the exposure chambers ranged from 21-22°C. The overall mean relative humidity in each of the exposure chambers ranged from 35-55%, and the oxygen concentration was approximately 21 %. The mean chamber airflows ranged from 320-330 L/min in the 1.4-m³ control chambers and 1600-1800 L/min in the 9-m³ test chambers.</p> <p>No compound-related effects were observed on mortality, clinical signs of toxicity, clinical pathology,</p>

neurobehavioral endpoints, neuropathology, sperm motility or morphology, or estrous cycle.

Male rats exposed to 400 mg/m³ DMG had lower mean body weights and mean body weight gains during the study. In addition, male and female rats exposed to 400 mg/m³ DMG had lower food consumption.

Compound-related effects were observed in the noses of male and female rats exposed to 400 mg/m³ of DMG for 90 days. These effects consisted primarily of degeneration/atrophy of the olfactory mucosa of the dorsal meatus and of the dorsomedial aspect of the dorsal endoturbinate. Less commonly, focal respiratory metaplasia of the olfactory mucosa of the dorsal meatus was also present. Lesions were minimal to mild in severity, did not occur below the 400 mg/m³ exposure level and occurred in higher incidences in the DMG group. Degeneration/atrophy of the olfactory mucosa occurred in recovery animals in the same locations as was apparent at the 90-day sacrifice in animals exposed to DMG. The lesions were usually focal and minimal in severity.

Male rats exposed to 400 mg/m³ DMG had significantly greater CP in the nose level III at day 90. CP in the nose level III of female rats exposed to 400 mg/m³ DMG was significantly greater than controls on day 14. Female rats exposed at 400 mg/m³ DMS had significantly greater CP in the nose level III compared to controls on day 90.

In male rats exposed to DMG, serum testosterone concentrations were statistically significantly decreased at concentrations of 50 and 400 mg/m³ (59 and 50% of control, respectively). Similarly, serum LH concentrations were decreased in a dose-dependent manner and were statistically significantly decreased at 400 mg/m³ (71% of control). Serum concentrations of FSH were not affected by DMG treatment. In female rats, DMG exposure did not alter serum estradiol or progesterone concentrations.

There was a treatment-related increase in epididymal sperm counts (per cauda epididymis and per gram cauda epididymis) following exposure to DMG and the number of sperm per cauda and per gram cauda epididymis was significantly increased at 50 and 400 mg/m³ (124-131% of control). Epididymal sperm counts were similar to control at 10 mg/m³ DMG. Under the conditions of this study, the no-observed-effect level (NOEL) for repeated exposure to

	DMG was 10 mg/m ³ , based on the decreases in serum testosterone and serum LH concentrations and increased epididymal sperm counts at concentrations of 50 mg/m ³ and above.
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Dupont Co. (2000). Unpublished data, Haskell Laboratory: MR-13128-1, Dupont-3557.
Remarks:	The NOEL for this study is defined as the highest dose at which toxicologically important effects attributable to the test substance were not detected. Thus, for this study, the NOEL is equivalent to the NOEL as defined by the United States Environmental Protection Agency (1985) and to the no-observed-adverse-effect level (NOAEL) as defined by the European Union (1994).

5.3. Developmental Toxicity

No information was found

5.4. Reproductive Toxicity

Type:	90-day
Guideline:	Based on 40CFR799.9346 and 799.9380
Species/Strain:	Rats/ CrI:CD@(SD)IGS BR
Sex/Number:	36 male/36 female per treatment level
Route of Administration:	inhalation
Exposure Period:	90 days
Frequency Of treatment:	6 hours/day, 5 days/wk
Exposure levels:	0 or 400 mg/m ³ DMG
Methods:	Male and female rats were exposed via inhalation to 0, 10, 50, or 400 mg/m ³ dimethyl glutarate (DMG) over a 90-day period. The exposure period was followed by a one-month recovery period. Approximately 90 days after study initiation, rats in the clinical pathology subgroups were sacrificed and evaluated for sperm motility, sperm number, and sperm morphology. The estrous cycle of female rats was determined for the last 21 days of exposure. Hormonal analyses were conducted following 90 days of exposure. Serum LH, FSH, and testosterone concentrations were measured in the male rats and serum estradiol and progesterone concentrations were measured in the female rats.

GLP:	Yes
Test Substance:	99.61% DMG
Results:	No concentration-related effects were observed on sperm motility or morphology or estrous cycle. In female rats, DMG exposure did not alter serum estradiol or progesterone concentrations. In male rats exposed to DMG, serum testosterone concentrations were significantly decreased at concentrations of 50 and 400 mg/m ³ (59 and 50% of control, respectively). Similarly, serum LH concentrations were decreased in a dose-dependent manner and were significantly decreased at 400 mg/m ³ (71% of control). Serum concentrations of FSH were not affected by DMG treatment. There was a treatment-related increase in epididymal sperm counts (per cauda epididymis and per gram cauda epididymis) following exposure to DMG and the number of sperm per cauda and per gram cauda epididymis was significantly increased at 50 and 400 mg/m ³ (124-131% of control). Epididymal sperm counts were similar to control at 10 mg/m ³ .
Reliability:	High (Scientifically defensible, or guideline method, GLP)
Reference:	Dupont Co. (2000). Unpublished data, Haskell Laboratory: MR-13128-1, Dupont-3557.
Remarks:	Additional details of this study can be found in the sub chronic inhalation section (DuPont Co., 2000).

5.5. Genetic Toxicity *in vitro* (gene mutations)

Type:	<i>In vitro</i> Mouse Lymphoma Forward Mutation Bioassay
Tester strains:	Mouse lymphoma cells (L5178Y;TK locus)
Exogenous	
Metabolic	
Activation:	With and without activation.
Exposure	
Concentrations:	No Data
Methods:	No Data
GLP:	No
Reliability:	Not assignable because limited study information was available.
Reference:	Bradford, J.C. et al. (1984). <u>Teratology</u> , 29(2):19A.
Remarks:	The results of this assay were dependent upon the pH of the culture medium. Evidence suggested that the variable response may not be a direct effect of gluteric acid, per se, but rather an indirect effect of other factors (i.e., pH, osmolality) of the media in which the cells were exposed.

Additional References for *in vitro* Studies:

Sakagami, Y. et al. (1988). Mutat. Res., 209(3-4):155-160. [Gluteric acid did not induce umu gene expressions independently or in the presence of S-9 fraction.]

5.6. Genetic Toxicity *in vivo* (chromosomal aberrations)

Guideline:	US EPA (1998). Health Effects Test Guidelines. OPPTS 870.5395
Type:	Rat Micronucleus Test
Cell Type:	Fischer 344 rat bone marrow cells (immature erythrocytes)
Route of Administration:	Inhalation
Exogenous Metabolic Activation:	None
Exposure Concentrations:	0.5, 1.0 and 2.0 mg/L (w/v) of chamber air
Method:	Ten Fischer 344 rats, six to eight weeks old, were exposed (5 male/5 female) to each of three exposure levels: 0.5, 1.0 and 2.0 mg/L DMG (w/v) via inhalation. Two six-hour exposures on consecutive days were used for all animals including negative controls. Test material used as received. A negative inhalation control (air only) and a positive control consisting of oral gavage of cyclophosphamide were employed. Following a period of 24 hours post-exposure animals were sacrificed and immature erythrocytes in bone marrow smears (one smear from each animal exposed) were examined for micronuclei.
GLP:	Yes
Test Substance:	99.6% DMG
Results:	No statistically significant increase in micronucleated, immature erythrocytes ($P>0.01$) or significant decrease in immature erythrocytes ($P>0.01$) was observed in rats exposed to DMG by inhalation.
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Huntington Life Sciences, Ltd. (2001). "Dimethyl Glutarate Rat Micronucleus Test". Submitted to SOCMA. SOA 001/004850.

Additional References for *in vivo* Studies:

Bradford, J.C. et al. (1984). Teratology, 29(2):19A.

6.0 Other Information

6.1 Biochemical/Metabolism Studies

Hepatic mitochondria were used as a model for nasal tissue. DBEs were found to inhibit mitochondrial ATP synthesis 11 to 27% at 100 μ M. The order of potency was DMA > DMG > DMS and paralleled the V_{\max}/K_m values for the hydrolysis of the DBEs to their monomethyl esters. Pre-treatment of the rats with 100 mg/kg of bis-nitrophenyl phosphate for three days decreased the rate of hydrolysis of the DBEs approximately 50% and protected the mitochondria from DBE-induced inhibition of ATP synthesis. These results support the hypothesis that DBE-induced cytotoxicity results from esterase-mediated hydrolysis to acid metabolites and interference with intermediary metabolism (Bogdanffy and Londergan, 1989).

In the study cited above, DBE cytotoxicity was shown to be due to esterase-mediated activation. In this present study, the putative toxic monomethyl and diacid metabolites were evaluated in an *in vitro* nasal explant system. Monomethyl adipate (MMA), glutarate (MMG), and succinate (MMS) induced increases in nasal explant acid phosphatase release, a biochemical index of their cytotoxicity. Metabolism of MMA and MMG to their diacids paralleled cytotoxicity. MMS metabolism was not quantifiable. Pretreatment of rats with a carboxylesterase inhibitor reduced cytotoxicity and metabolism of MMA and MMG, but not cytotoxicity of MMS. It is concluded that both monomethyl ester and diacid metabolites of DBE are cytotoxic. The contribution of each to cytotoxicity *in vivo* may depend on their rate of formation during exposure (Bogdanffy et al., 1991a; Trela and Bogdanffy, 1991b).

The kinetic parameters V_{\max} , K_m , K_{si} , and V/K were measured for the hydrolysis of the dibasic esters in the target nasal tissue, olfactory mucosa, and non-target tissue, respiratory mucosa. It was determined under the conditions of these experiments, diacid metabolites were not formed. Esterase activity was inhibited by pretreatment with bis-nitrophenyl phosphate. V_{\max} values for the three dibasic esters were 5- to 13-fold greater in olfactory mucosa than respiratory mucosa for male and female rats. V/K values were 4- to 11-fold greater in olfactory mucosa than respiratory mucosa for male and female rats. V/K was similar between male and female olfactory mucosa when DMG was used as the substrate. With DMS or DMA as the substrate, V/K for female olfactory tissue was 0.5- or 2-fold that of males, respectively. Differences in V/K were mainly due to decreases in K_m associated with increasing carbon chain length. Substrate inhibition was observed at DBE concentrations greater than approximately 25 mM, which are unlikely to be achieved *in vivo*. These results lend further support to the hypothesis that organic acid accumulation in the target tissue, olfactory mucosa, plays a significant role

in the pathogenesis of DBE-induced nasal lesions (Bogdanffy et al., 1991b).

Since female rats appear to be more sensitive to DBE-induced olfactory toxicity than males, it was of interest to measure the rate of hydrolysis of DBEs in male and female nasal mucosa homogenates and compare these values to those derived from human nasal tissue obtained at autopsy. For both male and female rats, V_{\max}/K_m values followed the order DMA > DMG > DMS paralleling carbon chain length. The V_{\max}/K_m values for female olfactory mucosa using DMA or DMS as substrates were two times or one-half the values for male olfactory mucosa, respectively. Hydrolysis of DBEs was detectable in only three of six human samples. Activity values that were measurable were two or three orders of magnitude lower than that of rat respiratory or olfactory mucosa, respectively. These data suggest the rate of conversion of DBEs to acid metabolites in nasal tissue is less significant in humans than in rats, and that the rat may be more sensitive than man to the effects of DBEs on nasal mucosa (Kee et al., 1989).

The enzymatic esterase activity of carboxylesterases is integral to the nasal toxicity of many esters, including DMG, DMS, and DMA. Inhalation of these esters specifically damages the olfactory mucosa of rodents. In this study, the localization differential distribution of a 59 KD carboxylesterase was demonstrated in the nasal tissues of the rat by immunohistochemistry. Rabbit antiserum against the 59 KD rat liver microsomal carboxylesterase bound most prominently to the olfactory mucosa when applied to decalcified, paraffin-embedded sections of rat nasal turbinates. Within the olfactory mucosa, anti-carboxylesterase did not bind to sensory neurons, the target cell for ester-initiated toxicity; these cells apparently lack carboxylesterase. Instead, the antibody was preferentially bound by cells of Bowman's glands and sustentacular epithelial cells that are immediately adjacent to the olfactory nerve cells. In contrast, non-olfactory tissues (respiratory mucosa and squamous epithelium) which are more resistant to the toxicity of esters, had less carboxylesterase content (Olson et al., 1993).

An *in vitro* system was utilized to determine if DBE toxicity is dependent on metabolic activation by carboxylesterase. Explants from the olfactory and respiratory regions of the rat nasal cavity were incubated in a medium containing 10-100 mM of the dimethyl esters of adipic-, glutaric-, and succinic acids. DBE caused a dose-related increase in nasal explant acid phosphatase release, a biochemical index of cytotoxicity. A parallel increase in carboxylesterase-mediated monomethyl ester (MME) formation was seen. In addition, MME concentrations and acid phosphatase release were generally higher in olfactory than respiratory tissues. DME-induced cytotoxicity and MME formation were markedly reduced in nasal tissue excised from rats treated with a carboxylesterase inhibitor, bisnitrophenyl phosphate (Trela and Bogdanffy, 1990; 1991a).

The kinetic constants were determined for carboxylesterase-mediated hydrolysis of DBEs and correlated with lesion formation. No diacid metabolites were found. V max values for the formation of MMS, MMG, and MMA were approximately 8- to 10-times larger in olfactory mucosa than in respiratory mucosa. V/K values for the formation of MMG and MMA were approximately 9- and 10-times larger in olfactory mucosa than respiratory mucosa. For the formation of MMS, V/K was approximately 2 times larger in respiratory mucosa than olfactory mucosa (Patterson et al., 1988).

To determine the biochemical mechanism for the toxic effect of DBE on rat nasal olfactory mucosa, an *in vitro* study was conducted with rat and human nasal tissue. This study demonstrated that the nasal tissue toxicity of DBE is related to enzymatic hydrolysis of DBE within the nasal cavity to form the corresponding monomethyl ester. Additionally, it was found that human nasal tissue hydrolyzes DBE at 1/100 to 1/1000 the rate of rat nasal tissue. For this reason, the nasal tissue of humans is likely to be at greatly reduced risk of DBE toxicity compared to rats (Bogdanffy and Frame, 1994).

- References:
- Bogdanffy, M. S. and T. Londergan (1989). The Toxicologist, 9(1):249 (Abstract 996).
- Bogdanffy, M. S. et al. (1991a). The Toxicologist, 11(1):182 (Abstract 664).
- Bogdanffy, M. S. et al. (1991b). Drug Metab. Dispos. Biol. Fate Chem., 19(1):124-129.
- Bogdanffy, M. S. and S. R. Frame (1994). Inhalation Toxicology, 6(Supplement):205-: (NIOSH/00224297) (as cited in Dupont Co. (1995). AEL Documentation, October 4..
- Kee, C. R. et al. (1989). The Toxicologist, 9(1):284 (Abstract 1139).
- Olson, M. J. et al. (1993). J. Histochem. Cytochem., 41(2):307-311(BIOSIS/93/13203).
- Patterson, C. A. et al. (1988). The Toxicologist, 8(1):6 (Abstract 22).
- Trela, B. A. and M. S. Bogdanffy (1990). The Toxicologist, 10(1):261 (Abstract 1044).

Robust Summaries for Dibasic Ester Solvents: Dimethyl Adipate (DMA)

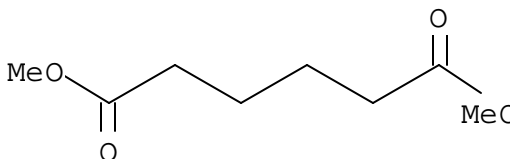
1. Substance Information

1.1. Chemical Name: Dimethyl Adipate

1.2. CAS Registry No: 627-93-0

1.3. Component CAS Nos.: Not applicable

1.4. Structural Formula:



1.5. Other Names: Hexanedioic acid, dimethyl ester, dimethyl hexanedioate, dibasic acid ester (dimethyl adipate), dimethyl adipate, 1,4 butanedicarboxylic acid, dibasic dimethyl ester of adipic acid, DMAD and DBE-6.

2. Physical-Chemical Properties

2.1. Melting Point

Value: 8.5 °C

Decomposition: No Data

Sublimation: No Data

Method: No Data

GLP: No Data

Reliability: Not assignable because limited study information was available

Reference: IUCLID (2000). IUCLID Dataset. European Chemicals Bureau, European Commission. Datasheet for dimethyl adipate, 2/18/00 [Subsequently referenced as IUCLID (2000)]

Additional References for Melting Point:

Dupont Co. (2001). Material Safety Datasheet DU005940 for DBE-6 (dimethyl adipate, DMA).

HSDB (2001). Hazardous Substances Data Bank (HSDB/5021)

2.2. Boiling Point

Value:	230.9 °C
Decomposition:	No Data
Pressure:	1013 hPa
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	IUCLID (2000)

Additional References for Boiling Point:

Dupont Co. (2001). Material Safety Datasheet DU005940 for DBE-6 (dimethyl adipate, DMA).

Lide , D.R. (ed.) (1995-1996). CRC Handbook of Chemistry and Physics. 76th ed., p. 3-187, CRC Press, Inc. Boca Raton, FL.

2.3. Density

Value:	1.062 g/cm ³
Temperature:	20°C
Method:	No Data
GLP:	No Data
Reference:	IUCLID (2000)
Reliability:	Not assignable because limited study information was available

Additional References for Density:

Dupont Co. (2001). Material Safety Datasheet DU005940 for DBE-6 (dimethyl adipate, DMA).

Lide , D.R. (ed.) (1995-1996). CRC Handbook of Chemistry and Physics. 76th ed., p. 3-187, CRC Press, Inc. Boca Raton, FL.

2.4. Vapor Pressure

Value:	0.17 hPa
Temperature:	20°C
Decomposition:	No Data
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	IUCLID (2000)

Additional References for Vapor Pressure:

Dupont Co. (2001). Material Safety Datasheet DU005940 for DBE-6 (dimethyl adipate, DMA).

2.5. Partition Coefficient (log K_{ow})

Value: 1.03 (calculated)
Reliability: Not assignable because limited study information was available
Reference: Hansch, C., A. Leo, D. Hoekman (1995). Exploring QSAR-Hydrophobic, Electronic, and Steric Constants. Washington, D.C., American Chemical Society (HSDB 5021).

Additional References for Partition Coefficient:

IUCLID (2000)

2.6. Water Solubility

Value: 29.9 g/L
Temperature: 20°C
pH/Pka: pH 8.9
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available
Reference: IUCLID (2000)
Remarks: Another reference reports a lower level of solubility of 600 mg/L with temperature not specified (Bennet SR et al (1984). "Environmental Hazards of Chemical Agent Stimulants". Aberdeen Proving Ground, MD: CRDC-TR-84055.

Additional References for Water Solubility:

Dupont Co. (2001). Material Safety Datasheet DU005940 for DBE-6 (dimethyl adipate, DMA).

2.7. Flashpoint

Value: 116 °C
Type: Closed cup
Method: other: DIN 51794
Reliability: Not assignable because limited study information was available
Reference: IUCLID (2000)

Additional References for Flashpoint:

Dupont Co. (2001). Material Safety Datasheet DU005940 for DBE-6 (dimethyl adipate, DMA).

3. Environmental Fate

3.1. Photodegradation

Value: 50% degradation after 5 days with rate constant of 3.221×10^{-12} molecules /second.

Indirect Photolysis: In air

Breakdown

Products: No Data

Method: Calculated using AOP computer program, Version 1.53, Syracuse Research Center (SRC). Used concentration of sensitizer (OH) of 500,000 molecules/cm³.

GLP: Not Applicable

Reliability: Estimated value based on accepted model

Reference: Adkinson, R. (1987). Int. J. Chem. Kinet., 19:799-828 (cited in IUCLID.(2000).)

Remarks: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere (Bidleman, 1988), dimethyl adipate, which has a vapor pressure of 0.06 mm Hg at 25°C (Howard and Meylan, 1997), is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase dimethyl adipate is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals (SRC, n.d.); the half-life for this reaction in air is estimated to be four days (SRC, n.d.), calculated from its rate constant of 4×10^{-12} cm³/molecule-sec at 25°C (Meylan and Howard, 1993). Dimethyl adipate may undergo direct photolysis in the environment, since this compound contains a functional group that can absorb light >290 nm (Lyman et al., 1990) (HSDB/5021).

Additional References for Photodegradation:

Bidleman, T.F. (1988). Environ. Sci. Technol. 22:361-367 (HSDB/5021).

Meylan, W.M., and P.H. Howard. (1993). Chemosphere 26:2293-99 (HSDB/5021).

Howard, P.H. and W.M. Meylan. (1997). Handbook of Physical Properties of Organic Chemicals. Lewis Publ. Boca Raton, FL: (HSDB/5021).

Lyman, W.J., et al.(1990). Handbook of Chemical Property Estimation Methods Am. Chem. Soc., Washington, DC. (HSDB/5021).

3.2. Stability in Water

Concentration: No Data

Half-life: Hydrolytic half-life is estimated as 2 years and 60 days, respectively (calculated), at pH 7 and 8.

Percent Hydrolyzed: Not applicable

Method: SAR based calculation.

GLP: No

Reliability: Estimated value based on accepted model

Reference: Mill, T., et al. (1987). Environmental Fate and Exposure Studies Development of a PC-SAR for Hydrolysis: Esters, Alkyl, Halides and Epoxides. SRI International, Menlo Park, CA. EPA Contract No. 68-02-4254 (HSDB/5021).

Remarks: Based on a classification scheme (Swann et al., 1983), a Koc value of 11 (SRC, n.d.), determined from an estimation method (Meylan et al., 1992), indicates that dimethyl adipate is not expected to adsorb to suspended solids and sediment in water (SRC, n.d.). Volatilization from water surfaces is not expected (Lyman et al., 1990) based upon an estimated Henry's Law constant of 9.77×10^{-7} atm-m³/mole (Meylan and Howard 1991), developed using a fragment constant estimation method (Meylan and Howard, 1991). According to a classification scheme (Franke et al., 1994), an estimated BCF of 1.2 (SRC, n.d.), from a log Kow of 1.03 (Hansch et al., 1995) and a regression-derived equation (Meylan et al., 1999), suggests the potential for bioconcentration in aquatic organisms is low. Dimethyl adipate is expected to undergo hydrolysis producing hexanedioic acid and methanol (SRC, n.d.). Estimated hydrolysis half-lives are two years and 60 days at pH values of 7 and 8, respectively (Mill et al.; 1987) (HSDB/5021).

Additional References for Stability in Water:

- Bidleman, T.F. (1988). Environ. Sci. Technol. 22:361-367 (HSDB/5021).
- Franke, C. et al. (1995). Chemosphere, 29:1501-14 (HSDB/5021)
- Hansch, C. et al. (1995). Exploring QSAR. Hydrophobic, Electronic and Steric Constants, p. 48, Amer. Chem. Soc., Washington, DC (HSDB/5021).
- Howard, P.H. and W.M. Meylan. (1997). Handbook of Physical Properties of Organic Chemicals. Lewis Publ. Boca Raton, FL: (HSDB/5021).
- Lyman, W.J., et al. (1990). Handbook of Chemical Property Estimation Methods. Am. Chem. Soc., Washington, DC. (HSDB/5021).
- Meylan, W.M., and P.H. Howard. (1993). Chemosphere 26:2293-99 (HSDB/5021).
- Meylan, W.M. et al. (1999). Environ. Toxicolo. Chem., 18:664-72 (HSDB/5021)

Mill, T. et al. (1987). Environmental Fate and Exposure Studies Development of a PC-SAR for Hydrolysis: Esters, Alkyl Halides and Epoxides, EPA Contract No.68-02-4254, SRI International, Menlo Park, CA (HSDB/5021).

SRC (n.d.) Syracuse Research Corporation (HSDB/5021).

Swann, R. L. et al. (1983). Res. Rev., 85:17-28 (HSDB/5021).

3.3. Transport (Fugacity)

Media:	Air, water, soil, and sediment	
Distributions:	Air	4.13%
	Water	50.7%
	Soil	45.1%
	Sediment	0.0923%
Adsorption		
Coefficient:	Not applicable	
Desorption:	Not applicable	
Volatility:	Not applicable	
Method:	Calculated method based on Mackay's Level III Fugacity model and using Estimations Programs Interface (EPIWIN v. 3.10)	
Data Used:		
	Molecular Weight:	174.2
	Henry's Law Constant:	9.77 x 10 ⁻⁶ atm.-m ³ /mole (calculated VP/Wsol)
	Vapor Pressure:	1.27 mm Hg (user -entered)
	Log Kow:	1.03 (KOWWIN Program).
	Soil Koc:	4.39 (calculated by model)
GLP:	No	
Reliability:	Estimated value based on accepted SAR model.	
Reference:	SRC and EPA developed models EPIWIN (v. 3.10) which contains a Level III Fugacity Model developed by Dr. Donald MacKay and Co-Workers which is detailed in: Mackay, D. (1991). <u>Multimedia Environmental Models: the Fugacity Approach</u> . pp 67-183, Lewis Publishers, CRC Press. MacKay, D. et al. (1996) <u>Environ. Toxicol. Chem.</u> , 15(9):1618-1626. MacKay, D. et al. (1996). <u>Environ. Toxicol. Chem.</u> , 15(9):1627-1637.	

3.4. Biodegradation

Value:	Modeled Linear Biodegradation Probability 1.0130
Breakdown	
Products:	Not applicable
Method:	Calculated using BIOWIN (v. 4.00) developed for the EPA by Syracuse Research Center (SRC) (© 2000 US EPA).
GLP:	No
Reliability:	Estimated value based on accepted SAR model.
Reference:	Boethling, R.S., Howard, P.H., Meylan, W.M., Stiteler, W., Beauman, J. and Tirado, N. 1994. Group contribution method for predicting probability and rate of aerobic biodegradation. Environ. Sci. Technol. 28: 459-65. (BIOWIN Software available from Syracuse Research Corp, Environmental Science Center, Syracuse, NY 13210). Howard, P.H., Boethling, R.S., Stiteler, W., Meylan, W.M., Hueber, A.E., Beauman, J. and Larosche, M.E. 1992. Predictive model for aerobic biodegradability developed from a file of evaluated biodegradation data. Environ. Toxicol. Chem. 11: 593-603. (BIOWIN Software available from Syracuse Research Corp, Environmental Science Center, Syracuse, NY 13210).

3.5. Bioconcentration

Value:	BCF of 1.2 (calculated)
Method:	An estimated BCF of 1.2 was calculated for dimethyl adipate (SRC, n.d.), using a log Kow of 1.03 (Hansch et al., 1995) and a regression-derived equation (Meylan et al., 1999). According to a classification scheme (Franke et al., 1994), this BCF suggests the potential for bioconcentration in aquatic organisms is low (HSDB/5021).
GLP:	No
Reliability:	Not assignable because limited study information was available
Reference:	HSDB/5021

Additional References for Bioconcentration:

- Franke, C. et al. (1995). Chemosphere, 29:1501-14 (HSDB/5021)
- Hansch, C. et al. (1995). Exploring QSAR. Hydrophobic, Electronic and Steric Constants, p. 48, Amer. Chem. Soc., Washington, DC (HSDB/5021).
- Meylan, W.M. et al. (1999). Environ. Toxicolo. Chem., 18:664-72 (HSDB/5021)
- SRC (n.d.) Syracuse Research Corporation (HSDB/5021).

4. Ecotoxicity

4.1. Acute Toxicity to Fish

Type:	43-h LC ₅₀
Species:	<u>Carpus carpio</u> (Carp)
Value:	No mortality reported
Method:	Carp were exposed to 89-122 mg/L for 43 hours. Water temperature was 12°C and dissolved O ₂ was 8.5 –9.5 mg/L.
Results:	A 5-17% hematoma incidence was reported.
GLP:	No
Reliability:	Not assignable because limited study information was available.
Reference:	Loeb, H.A. et al. (1963). U.S. Fish Wildl. Serv. Sp. Sci. Rep.-Fish No. 471, Washington, DC (Aquire/AQ-0002965).

Additional References for Fish Toxicity:

The aquatic toxicity of DMA was estimated using quantitative structure-activity relationships. The estimated LC₅₀ was 27 mg/L; the estimated lowest chronic EC₅₀ was 6.74 mg/L; the estimated chronic “safe level” for aquatic life was 270-674 µg/L; and the calculated K_{ow} was 0.36.

Matthiessen, P. et al., (1993). Mar. Poll. Bull., 26(2):90-95

4.2. Acute Toxicity to Invertebrates

Type:	48-h LC ₅₀
Species:	<u>Daphnia magna</u>
Value:	497 mg/L
Method:	Calculated using ECOSAR (v. 0.99g) developed for the EPA by Syracuse Research Center (SRC) (© 2000 US EPA).
GLP:	No
Reliability:	Estimated value based on accepted SAR model.
Reference:	Meylan, W.M. and P.H. Howard. (1999). User's Guide for the ECOSAR Class Program. Version 0.99e (Mar 1999), prepared for J. Vincent Nabholz and Gordon Cash, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, North Syracuse, NY 13212.

4.3. Acute Toxicity to Aquatic Plants

Type: 96-h EC50
Species: Green algae
Value: 4.351 mg/L
Method: Calculated using ECOSAR (v. 0.99g) developed for the EPA by Syracuse Research Center (SRC) US EPA).
GLP: No
Reliability: Estimated value based on accepted SAR model.
Reference: Meylan, W.M. and P.H. Howard. (1999). User's Guide for the ECOSAR Class Program. Version 0.99e (Mar 1999), prepared for J. Vincent Nabholz and Gordon Cash, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, North Syracuse, NY 13212.

5. Mammalian Toxicity

5.1. Acute Toxicity

Type: Acute Oral Toxicity
Guideline: DOT Protocol
Value: 7500 mg/kg b.wt.
Methods: Using a Department of Transportation (DOT) protocol 10 rats were administered (orally) 50 mg/kg b. wt. DMA
GLP: No
Test Substance: DMA
Results: No mortalities were observed at this 50 mg/kg dose level, indicating that DMA is not a Class B poison
Reliability: High (Scientifically defensible or guideline method)
Reference: Dupont Co. (1966). Unpublished data, Haskell Laboratory Report No.149-66.

Type: Oral LD50
Guideline: EPA (40 CFR) 798.1175
Species/Strain: Sprague Dawley Rats
Value: >5000 mg/kg b.wt.
Method: DMA was administered a single dose via oral gavage at two dose levels (500 and 5000 mg/kg b.wt.) with a 14 day observation period. Test material was administered as received. 5 females and 5 males were used for each dose level. Necropsies were performed on animals that died and that survived to 14 days post-administration.
GLP: Yes
Test Substance: 99.5% DMA

Results:	At 500 and 5,000 mg/kg b.wt. mortality rates were 0/10 and 2/10, respectively. The mortalities occurred in the first two days. The animals in the 500 mg/kg b. wt. dose group were free of signs of abnormality. In the 5,000 mg/kg treatment level the only abnormality was decreased activity and “hunched” appearance. Other signs observed in single animals included irregular gait. Surviving animals showed no abnormalities with the exception of a single animal with red foci in the lungs. Necropsies of two dead animals (i.e., 5,000 mg/kg dose group) revealed discoloration and edema in the lungs, discoloration of the thymus and the presence of thick tarry substance in the stomach. Other observations in individual animals were reddened pancreas and yellow fluid in the stomach.
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Bio/dynamics Inc. (1992). Acute Oral Toxicity in Rats (DMA, Dimethyl Adipate). Submitted to Monsanto Company. Reference Numbers: 92-6328, BD-92-243, October 8, 1992.
Remarks:	Study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture of three dimethyl esters), DMA, DMG, and DMS.

Additional References for Acute Oral Toxicity:

Dupont Co. (1975). Unpublished data, Haskell Laboratory Report No.34-75.

Type:	Dermal LD50
Guideline:	EPA (40 CFR) 798.1100
Species/Strain:	New Zealand White Rabbits
Value:	> 5000 mg/kg b. wt.
Method:	DMA was administered a single dose applied directly to the skin over a 12 x 14 cm area approximating 10% of the body surface. Contact with excess material was maintained for 24 hours and animals were observed for 14 days after initiation of dose. A total of 5 females and 5 males were used for this study. Test material was administered as received.
GLP:	Yes
Test Substance:	99.5% DMA
Results:	All animals survived after dermal treatment at 5,000 mg/kg b. wt. Animals gained weight during 7 days post-treatment, but all animals lost weight or remained stable from day 7 to 14 post-treatment. All animals were free of systemic toxicity throughout the study and no abnormalities were observed during post-mortem macroscopic observation.

Reliability: High (Scientifically defensible or guideline method, GLP)
Reference: Bio/dynamics Inc. (1992). Acute Dermal Toxicity in Rabbits (DMA, Dimethyl Adipate). Submitted to Monsanto Company Reference Numbers: 92-6329, BD-92-243, October 8, 1992.
Remarks: Study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture of three dimethyl esters), DMA, DMG, and DMS.

Additional References for Acute Dermal Toxicity:

Dupont Co. (1975). Unpublished data, Haskell Laboratory Report No. 301-75.

Type: **Primary Dermal Irritation**
Guideline: **EPA (40 CFR) 798.4470**
Species/Strain: New Zealand White Rabbits
Value: Average Dermal Irritation Score (ADIS) was 0.0
Method: DMA was administered to six animals (3 female/3 male) as a single dose applied directly to two 1 x 1 inch areas of skin on the back and held in place with semi-occlusive dressings for 4 hours. Animals were observed for subsequent 3 days and treated areas were observed at 30 minutes and 24, 48, and 72 hours. Test material was administered as received.
GLP: Yes
Test Substance: 99.5% DMA
Results: The ADIS for DME is 0.0. No irritation was observed. This material would probably not be considered to produce dermal irritation as defined in EPA Guidelines.
Reliability: High (Scientifically defensible or guideline method, GLP)
Reference: Bio/dynamics Inc. (1992). Primary Dermal Irritation Study in Rabbits (DMA, Dimethyl Adipate). Submitted to Monsanto Company Reference Numbers: 92-6330, BD-92-243, October 8, 1992.
Remarks: Study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture of three dimethyl esters), DMA, DMG, and DMS.

Additional References for Dermal Irritation:

Dupont Co. (1975). Unpublished data, Haskell Laboratory Report No. 156-75.
When tested in rabbits resulted in mild irritation (grade 3 using a scale of 0-10).
Union Carbide Co. (1992). TSCA 8(e)CAP Submission, TSCA Fiche OTSO536615.

Type:	Eye Irritation
Guideline:	EPA (40 CFR) 798.4500
Species/Strain:	New Zealand White Rabbits
Value:	DMA produced mild to moderate, transient ocular irritation.
Method:	A single ocular administration of DMA (0.1 ml) was applied to 3 male and 3 female adult rabbits followed by observations at 1, 24, 48, 72 hours and 7 and 10 days. The observation period continued up to 10 days or until no signs of irritation were present. The cornea, iris and conjunctivae were observed and lesions were graded. Test material was administered as received.
GLP:	Yes
Test Substance:	99.5% DMA
Results:	DMA produced moderate, transient ocular irritation. This material would be considered to produce eye irritation as defined in the EPA Guidelines. All six animals exhibited moderate irritation of the conjunctivae (redness, chemosis, and discharge). Four animals exhibited corneal opacity, slight dulling of the corneal surface or had corneal ulceration and five animals exhibited iridial changes or damage. Two of the six animals were clear of irritation by 48 or 72 hours, and the remaining animals were free of irritation by Day 7.
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Bio/dynamics Inc. (1992). Primary Eye Irritation Study in Rabbits (DMA, Dimethyl Adipate). Submitted to Monsanto Company Reference Numbers: 92-6331, BD-92-243, October 8, 1992.
Remarks:	Study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture of three dimethyl esters), DMA, DMG, and DMS.

Additional References for Acute Eye Irritation:

Dupont Co. (1966). Unpublished data, Haskell Laboratory Report No. 196-66.

5.2. Repeated Dose Toxicity:

Type:	90-day Inhalation
Guideline:	Based on 40CFR799.9346, 799.9380, 799.9620, plus cell proliferation study.
Species/Strain:	Rats/ CrI:CD®(SD)IGS BR
Sex/Number:	36 male and 36 female/treatment level

Route of Administration:	inhalation
Exposure Period:	90 days
Frequency Of treatment:	6 hours/day, 5 days/wk
Exposure levels:	0 or 400 mg/m ³ DMA
Methods:	<p>Groups of male and female (nulliparous and non-pregnant) rats were exposed via inhalation to DMA over a 90-day period. The exposure period was followed by a 1-month recovery period. Rats were weighed once per week and clinical signs were taken daily. Food consumption was determined on a weekly basis. Samples for hepatic, lung, and nasal (levels II and III) cell proliferation (CP) were collected from rats approximately 2 weeks after initiation of the study and approximately 90 days after study initiation. A clinical pathology evaluation was conducted on rats approximately 45 and 90 days after initiation of the study. Approximately 90 days after study initiation, rats designated for the clinical pathology evaluation were sacrificed for pathological examination and evaluation of male reproductive endpoints, including sperm motility, sperm number, and sperm morphology. A neurobehavioral test battery, consisting of functional observational battery assessments and motor activity, was conducted prior to test substance administration to obtain baseline measurements, and during test weeks 4, 8, 13, and 18 (recovery). Rats designated for neuropathological evaluation were sacrificed approximately 90 days after study initiation and after approximately 1 month of recovery. The estrous cycle of female rats was determined for the last 21 days of exposure. Following 90 days of exposure, blood was collected via the tail vein from male and female rats and serum was subjected to hormonal analyses. In male rats, serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone concentrations were measured. In female rats, serum estradiol and progesterone concentrations were measured.</p>
GLP:	Yes
Test Substance:	98.824% DMA
Results:	<p>The analytically determined overall mean concentration of DMA in the exposure chambers targeted to 400 mg/m³ was 390 mg/m³. The overall mean temperature in each of the exposure chambers ranged from 21-22°C. The overall mean relative humidity in each of the exposure chambers ranged from 35-55%, and the oxygen concentration was</p>

approximately 21 %. The mean chamber airflows ranged from 320-330 L/min in the 1.4-m³ control chambers and 1600-1800 L/min in the 9-m³ test chambers.

No concentration-related effects were observed on mortality, clinical signs of toxicity, clinical pathology, neurobehavioral endpoints, neuropathology, sperm motility or morphology, estrous cycle, or serum hormone levels.

Male rats exposed to 400 mg/m³ DMA had lower mean body weights, lower mean body weight gains, and lower food efficiency during the study. Compound-related effects were observed in the noses of male and female rats exposed to 400 mg/m³ DMA for 90 days. These effects consisted primarily of degeneration/atrophy of the olfactory mucosa of the dorsal meatus and of the dorsomedial aspect of the dorsal endoturbinates. Less commonly, focal respiratory metaplasia of the olfactory mucosa of the dorsal meatus was also present. Lesions were minimal to mild in severity. Degeneration/atrophy of the olfactory mucosa occurred in recovery animals in the same locations as was apparent at the 90-day sacrifice. The lesions were usually focal and minimal in severity. Male rats exposed to 400 mg/m³ DMA showed significant increased CP in the liver at day 14 compared to controls and significantly greater CP in the nose level II at day 14. Female rats exposed to 400 mg/m³ DMA had significantly greater CP in the lungs relative to controls at days 14 and 90. Although not statistically significant, increased epididymal sperm counts were observed in the DMA group.

Reliability: High (Scientifically defensible or guideline method, GLP)
Reference: Dupont Co. (2000). Unpublished data, Haskell Laboratory: MR-13128-1, Dupont-3557.

Remarks: The NOEL for this study is defined as the highest dose at which toxicologically important effects attributable to the test substance were not detected. Thus, for this study, the NOEL is equivalent to the NOEL as defined by the United States Environmental Protection Agency (1985) and to the no-observed-adverse-effect level (NOAEL) as defined by the European Union (1994).

5.3. Developmental Toxicity

Species/Strain: Rats
Sex/Number: 5 rats/treatment
Route of

Administration: i.p. injection
 Exposure
 Period: 90 days
 Methods: Groups of five pregnant rats were administered 0.0603, 0.1809, 0.3617, or 0.6028 ml/kg/day of DMA (equivalent to 64, 192, 385, and 64 mg/kg/day) by i.p. injection on days 5, 10 and 15 of gestation.

GLP:
 Test Substance:
 Results:

	Resorptions	Abnormalities (percent)		
Dose	(percent)	Gross	Skeletal	Visceral
Control	6	0	3	0
64	6.8	0	0	0
192	14.1	1.8	7.4	0
385	1.8	3.6	13.8	0
641	5.7	8	19.2	8.3

No NOEL was reached in this study. In the 641 mg/kg/day group, 5/26 fetuses had elongated frontal ribs fused to the sternbrae. This group also contained two visceral abnormalities: one fetus had no left kidney and one fetus had an angulated anal opening. In the 385 mg/kg/day group, one fetus had no tail, 2/29 fetuses had a few elongated and fused posterior ribs, and two fetuses had elongated anterior ribs fused to the sternbrae. The 192 mg/kg/day group had one fetus with hemangioma of the right hind quarter while 2/27 fetuses had elongated anterior ribs fused to the sternbrae. Data concerning maternal toxicity were not available (Singh et al., 1973).

Reliability: Not assignable because limited study information was available
 Reference: Singh, R.L. et al. (1973). Res. Rev., 85:17-28 (HSDB/5021)

Additional References for Developmental Toxicity: None Found

5.4. Reproductive Toxicity

Type: 90-day
 Guideline: Based on 40CFR799.9346 and 799.9380
 Species/Strain: Rats/ CrI:CD@(SD)IGS BR
 Sex/Number: 36 male and 36 female/treatment level
 Route of Administration: Inhalation
 Exposure

Period:	90 days
Frequency	
Of treatment:	6 hours/day, 5 days/wk
Exposure levels:	0 or 400 mg/m ³ DMA
Method:	Male and female rats were exposed via inhalation to 0 or 400 mg/m ³ dimethyl adipate (DMA) over a 90-day period. The exposure period was followed by a one-month recovery period. Approximately 90 days after study initiation, rats in the clinical pathology subgroups were sacrificed and evaluated for sperm motility, sperm number, and sperm morphology. The estrous cycle of female rats was determined for the last 21 days of exposure. Hormonal analyses were conducted following 90 days of exposure. Serum LH, FSH, and testosterone concentrations were measured in the male rats and serum estradiol and progesterone concentrations were measured in the female rats.
GLP:	Yes
Test Substance:	98.824% DMA
Results:	No concentration-related effects were observed on sperm motility or morphology, estrous cycle, or serum hormone levels. Although not statistically significant, increased epididymal sperm counts were observed. Additional details of this study can be found in the subchronic inhalation section (Dupont Co. 2000).
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Dupont Co. (2000). Unpublished data, Haskell Laboratory: MR-13128-1, Dupont-3557.

Additional References for Reproductive Toxicity: None Found

5.5. Genetic Toxicity *in vitro* (gene mutations)

Type:	No Data
Tester strains:	No Data
Exogenous	
Metabolic	
Activation:	No Data
Exposure	
Concentrations:	No Data
Methods:	No Data
Reliability:	No Data
Reference:	No Data

5.6. Genetic Toxicity *in vivo* (chromosomal aberrations)

Type:	Rat Micronucleus Test
Cell Type:	Fischer 344 rat bone marrow cells (immature erythrocytes)
Route of Administration:	Inhalation
Exogenous Metabolic Activation:	None
Exposure Concentrations:	0.5, 1.0 and 2.0 mg/L (w/v)
Method:	This study followed test guidelines: US EPA (1998). Health Effects Test Guidelines, OPPTS 870.5395 Mammalian erythrocyte micronucleus test, EPA 712-C-98-226. Ten Fischer 344 rats, six to eight weeks old, were exposed (5 male/5 female) to each of three exposure levels: 0.5, 1.0 and 2.0 mg/L DMA (w/v) via inhalation. Two six hour exposures on consecutive days were used for all animals including negative controls. Test material used as received. A negative inhalation control (air only) and a positive control consisting of oral gavage of cyclophosphamide were employed. Following a period of 24 hours post-exposure animals were sacrificed and immature erythrocytes in bone marrow smears (one smear from each animal exposed) were examined for micronuclei.
GLP:	Yes
Test Substance:	98.8% DMA
Results:	No statistically significant increase in micronucleated, immature erythrocytes ($P > 0.01$) or significant decrease in immature erythrocytes ($P > 0.01$) was observed in rats exposed to DMA by inhalation.
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Huntington Life Sciences, Ltd. (2001). "Dimethyl Adipate Rat Micronucleus Test". Submitted to SOCMA 16 May 2001. SOA 001/004850.

Additional References for *in vivo*: None Found

6.0 Other Information

6.1 Biochemical/Metabolism Studies

Hepatic mitochondria were used as a model for nasal tissue. DBEs were found to inhibit mitochondrial ATP synthesis 11 to 27% at 100 μ M. The

order of potency was DMA > DMG > DMS and paralleled the V_{\max}/K_m values for the hydrolysis of the DBEs to their monomethyl esters. Pretreatment of the rats with 100 mg/kg of bis-nitrophenyl phosphate for three days decreased the rate of hydrolysis of the DBEs approximately 50% and protected the mitochondria from DBE-induced inhibition of ATP synthesis. These results support the hypothesis that DBE-induced cytotoxicity results from esterase-mediated hydrolysis to acid metabolites and interference with intermediary metabolism (Bogdanffy and Londergan, 1989).

In the study cited above, DBE cytotoxicity was shown to be due to esterase-mediated activation. In this present study, the putative toxic monomethyl and diacid metabolites were evaluated in an *in vitro* nasal explant system. Monomethyl adipate (MMA), glutarate (MMG), and succinate (MMS) induced increases in nasal explant acid phosphatase release, a biochemical index of their cytotoxicity. Metabolism of MMA and MMG to their diacids paralleled cytotoxicity. MMS metabolism was not quantifiable. Pretreatment of rats with a carboxylesterase inhibitor reduced cytotoxicity and metabolism of MMA and MMG, but not cytotoxicity of MMS. It is concluded that both monomethyl ester and diacid metabolites of DBE are cytotoxic. The contribution of each to cytotoxicity *in vivo* may depend on their rate of formation during exposure (Bogdanffy et al., 1991a; Trela and Bogdanffy, 1991b).

The kinetic parameters V_{\max} , K_m , K_{si} , and V/K were measured for the hydrolysis of the dibasic esters in the target nasal tissue, olfactory mucosa, and non-target tissue, respiratory mucosa. It was determined under the conditions of these experiments, diacid metabolites were not formed. Esterase activity was inhibited by pretreatment with bis-nitrophenyl phosphate. V_{\max} values for the three dibasic esters were 5- to 13-fold greater in olfactory mucosa than respiratory mucosa for male and female rats. V/K values were 4- to 11-fold greater in olfactory mucosa than respiratory mucosa for male and female rats. V/K was similar between male and female olfactory mucosa when DMG was used as the substrate. With DMS or DMA as the substrate, V/K for female olfactory tissue was 0.5- or 2-fold that of males, respectively. Differences in V/K were mainly due to decreases in K_m associated with increasing carbon chain length. Substrate inhibition was observed at DBE concentrations greater than approximately 25 mM, which are unlikely to be achieved *in vivo*. These results lend further support to the hypothesis that organic acid accumulation in the target tissue, olfactory mucosa, plays a significant role in the pathogenesis of DBE-induced nasal lesions (Bogdanffy et al., 1991b).

Since female rats appear to be more sensitive to DBE-induced olfactory toxicity than males, it was of interest to measure the rate of hydrolysis of DBEs in male and female nasal mucosa homogenates and compare these values to those derived from human nasal tissue obtained at autopsy. For

both male and female rats, V_{\max}/K_m values followed the order DMA > DMG > DMS paralleling carbon chain length. The V_{\max}/K_m values for female olfactory mucosa using DMA or DMS as substrates were two times or one-half the values for male olfactory mucosa, respectively. Hydrolysis of DBEs was detectable in only three of six human samples. Activity values that were measurable were two or three orders of magnitude lower than that of rat respiratory or olfactory mucosa, respectively. These data suggest the rate of conversion of DBEs to acid metabolites in nasal tissue is less significant in humans than in rats, and that the rat may be more sensitive than man to the effects of DBEs on nasal mucosa (Kee et al., 1989).

The enzymatic esterase activity of carboxylesterases is integral to the nasal toxicity of many esters, including DMG, DMS, and DMA. Inhalation of these esters specifically damages the olfactory mucosa of rodents. In this study, the localization differential distribution of a 59 KD carboxylesterase was demonstrated in the nasal tissues of the rat by immunochemistry. Rabbit antiserum against the 59 KD rat liver microsomal carboxylesterase bound most prominently to the olfactory mucosa when applied to decalcified, paraffin-embedded sections of rat nasal turbinates. Within the olfactory mucosa, anti-carboxylesterase did not bind to sensory neurons, the target cell for ester-initiated toxicity; these cells apparently lack carboxylesterase. Instead, the antibody was preferentially bound by cells of Bowman's glands and sustentacular epithelial cells that are immediately adjacent to the olfactory nerve cells. In contrast, non-olfactory tissues (respiratory mucosa and squamous epithelium) which are more resistant to the toxicity of esters, had less carboxylesterase content (Olson et al., 1993).

An *in vitro* system was utilized to determine if DBE toxicity is dependent on metabolic activation by carboxylesterase. Explants from the olfactory and respiratory regions of the rat nasal cavity were incubated in a medium containing 10- 100 mM of the dimethyl esters of adipic-, glutaric-, and succinic acids. DBE caused a dose-related increase in nasal explant acid phosphatase release, a biochemical index of cytotoxicity. A parallel increase in carboxylesterase-mediated monomethyl ester (MME) formation was seen. In addition, MME concentrations and acid phosphatase release were generally higher in olfactory than respiratory tissues. DME-induced cytotoxicity and MME formation were markedly reduced in nasal tissue excised from rats treated with a carboxylesterase inhibitor, bisnitrophenyl phosphate (Trela and Bogdanffy, 1990; 1991a).

The kinetic constants were determined for carboxylesterase-mediated hydrolysis of DBEs and correlated with lesion formation. No diacid metabolites were found. V_{\max} values for the formation of MMS, MMG, and MMA were approximately 8- to 10-times larger in olfactory mucosa than in respiratory mucosa. V/K values for the formation of MMG and

MMA were approximately 9- and 10-times larger in olfactory mucosa than respiratory mucosa. For the formation of MMS, V/K was approximately 2 times larger in respiratory mucosa than olfactory mucosa (Patterson et al., 1988).

To determine the biochemical mechanism for the toxic effect of DBE on rat nasal olfactory mucosa, an *in vitro* study was conducted with rat and human nasal tissue. This study demonstrated that the nasal tissue toxicity of DBE is related to enzymatic hydrolysis of DBE within the nasal cavity to form the corresponding monomethyl ester. Additionally, it was found that human nasal tissue hydrolyzes DBE at 1/100 to 1/1000 the rate of rat nasal tissue. For this reason, the nasal tissue of humans is likely to be at greatly reduced risk of DBE toxicity compared to rats (Bogdanffy and Frame, 1994).

- References:
- Bogdanffy, M. S. and T. Londergan (1989). The Toxicologist, 9(1):249 (Abstract 996).
- Bogdanffy, M. S. et al. (1991a). The Toxicologist, 11(1):182 (Abstract 664).
- Bogdanffy, M. S. et al. (1991b). Drug Metab. Dispos. Biol. Fate Chem., 19(1):124-129.
- Bogdanffy, M. S. and S. R. Frame (1994). Inhalation Toxicology, 6(Supplement):205-: (NIOSH/00224297) (as cited in Dupont Co. (1995). AEL Documentation, October 4..
- Kee, C. R. et al. (1989). The Toxicologist, 9(1):284 (Abstract 1139).
- Olson, M. J. et al. (1993). J. Histochem. Cytochem., 41(2):307-311(BIOSIS/93/13203).
- Patterson, C. A. et al. (1988). The Toxicologist, 8(1):6 (Abstract 22).
- Trela, B. A. and M. S. Bogdanffy (1990). The Toxicologist, 10(1):261 (Abstract 1044).

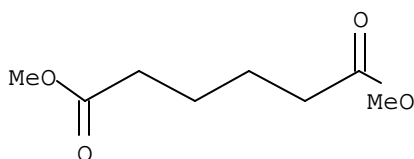
Robust Summaries for Dibasic Ester Solvents: Dibasic Ester Mixture (DBE)

1. Substance Information

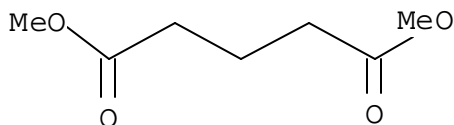
- 1.1. Chemical Name:** Dibasic Esters (DBE)¹
1.2. CAS Registry No: 95481-62-2
1.3. Component CAS Nos.: Dimethyl adipate: 627-93-0
Dimethyl glutarate: 1119-40-0
Dimethyl succinate: 106-65-0

1.4. Structural Formula:

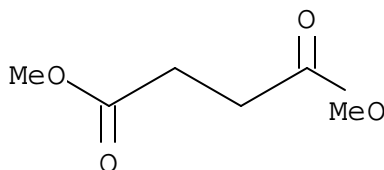
Dimethyl Adipate



Dimethyl Glutarate



Dimethyl Succinate



1.5. Other Names:

Estrasol, mixture of dimethyl adipate, dimethyl succinate, and dimethyl glutarate hexanedioic acid, dimethyl ester, mixture with dimethyl butanedioate and dimethyl pentanedioate, butanedioic acid, dimethyl ester mixture with dimethyl hexanedioate and dimethyl pentanedioate, dibasic acid ester (dimethyl succinate/glutarate/adipate), dimethyl succinate/glutarate/adipate, pentanedioic acid, dimethyl ester, mixture with dimethyl butanedioate and dimethyl hexanedioate, dibasic dimethyl esters

¹ DBE is a mixture of the dimethyl esters of adipic (10-25%), glutaric (55-75%), and succinic (19-26%) acids. There are also traces of methanol, water, and <10 ppm of hydrogen cyanide.

of adipic acid, succinic acid, and glutaric acid.

2. Physical-Chemical Properties

2.1. Melting Point

Value:	~ - 20°C
Decomposition:	No Data
Sublimation:	No Data
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	Dupont Co (2001). Material Safety Data Sheet FE000011

2.2. Boiling Point

Value:	196-225°C
Decomposition:	No Data
Pressure:	760 mm Hg
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available.
Reference:	Dupont Co (2001). Material Safety Data Sheet FE000011

2.3. Density

Value:	1.092
Temperature:	20°C
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	Dupont Co (2001). Material Safety Data Sheet FE000011

2.4. Vapor Pressure

Value:	0.2 mmHg
Temperature:	20°C
Decomposition:	No Data
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	Dupont Co. (2001). Material Safety Data Sheet FE000011

2.5. Partition Coefficient (log K_{ow})

Value:	0.19 (measured)
Temperature:	25°C
Decomposition:	No Data
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available.
Reference:	IUCLID (2000). IUCLID Dataset. European Chemicals Bureau, European Commission. Datasheet for dibasic esters, 2/18/00 [Subsequently referenced as IUCLID (2000)]

Additional References for Partition Coefficient

0.37 @ 25°C (calculated using CLOGP version 3.42). IUCLID (2000)

2.6. Water Solubility

Value:	5.3 wt% in water
Temperature:	20°
PH/Pka:	No Data
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	Dupont Co (2001). Material Safety Data Sheet FE000011

Additional References for Water Solubility

121-131 g/L @ 25°C. IUCLID (2000)

3. Environmental Fate

3.1. Photodegradation

Degradation:	Ca. 50% after 24.8 days
Rate Constant:	4.32×10^{-13} cm ³ /molecule/sec
Sensitizer:	OH
Method:	Calculated using ATMOSPHERIC OXIDATION PROGRAM (AOP), version 1.51 Syracuse Research Corporation.
GLP:	No Data

Reliability:	Not assignable because limited study information was available
Reference:	IUCLID (2000)

3.2. Stability in Water

Concentration:	No Data
Half-life:	No Data
Percent Hydrolyzed:	No Data
Method:	No Data
GLP:	No Data
Reliability:	No Data
Reference:	No Data

3.3. Transport (Fugacity)

Media:	No Data
Distributions:	No Data
Adsorption:	No Data
Coefficient:	No Data
Volatility:	No Data
Method:	No Data
GLP:	No Data
Reference:	No Data
Reliability:	No Data

3.4. Biodegradation

Type:	Shake Flask Method
Value:	Half-life ranged from 2.5 to 3.2 days
Method:	DBE was subjected to microbial biodegradation testing using the shake-flask method. The microbial inoculum was a combination of materials from three sources: Wilmington, Delaware secondary sewage treatment plant, filtrate from 300 g of forest soil, and inoculum from continually mixed microbial culture maintained in a biological digester. These inocula were combined in proportions of 4:2:1, respectively.
GLP:	No
Test Substance:	99% total dibasic esters (DBE)
Results:	The half-life for the high concentration of DBE (10 mg C/L) was 2.5 days while at the low DBE concentration (5 mg C/L), the half-life was 3.2 days. In both cases, 36% of the theoretical cumulative percent CO ₂ was respired by the mixed microbial system. The study included two external positive controls Proxel [®] and linear alkyl sulphonates (LAS) which showed less degradation in comparable system compared to DBE.
Reliability:	Moderate (scientifically defensible or guideline method, non-GLP)

Reference: Dupont Co. (1982). Unpublished Data,
Haskell Laboratory Report No. 698-82.

Additional References for Biodegradation:

IUCLID (2000)

3.5. Bioconcentration

Value: No Data
Method:
GLP:
Reference:
Reliability:

4. Ecotoxicity

4.1. Acute Toxicity to Fish

Type: 96-h LC₅₀
Species: *Pimephales promelas* (Fathead Minnow)
Value: >18 ppm (v/v) and <24 ppm (v/v)
Method: DBE toxicity to fathead minnows was evaluated using all-glass exposure chambers with 10 liters of water and 10 fish per chamber under static conditions. Exposures were monitored for temperature, pH, dissolved oxygen and other water quality parameters. The system was maintained as 16 hrs. light/ 8 hrs. dark photoperiod without aeration. Exposure levels were reported as nominal. Mortality counts and observations every 24 hours.
GLP: No
Reliability: Moderate (Scientifically defensible or guideline method, nominal concentrations, non GLP).
Reference: Dupont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 282-82.

Additional References for Acute Toxicity to Fish:

96-hr LC₅₀ 50-100 ppm (*Brachydanio rerio*). IUCLID (2000)

4.2. Acute Toxicity to Invertebrates

Type: 48-h EC₅₀ Study
Guideline: EPA. (1987). User's Guide: procedures for conducting *Daphnia magna* toxicity bioassays. EPA/600/8-87/011.

Species: *Daphnia magna* (Water Flea).
 Value: 48-h EC₅₀ is 136 ppm DBE (DME)
 Method: *Daphnia* were exposed to DBE (DME) for 48 hours under static conditions in replicate glass chambers with 250 ml volume with exposure concentrations ranging from 0 to 300 ppm. Test material was DME (lot # 192002) and additional data can be found in Lab Book # 4355288. Exposures were monitored for temperature, pH, dissolved oxygen and other water quality parameters. The system was maintained at 20°C and 24 hours light. Exposure levels were measured at initiation and at 48 hours.

GLP: Yes
 Results: 48-h EC₅₀ is 136 ppm DBE (DME), based on non-linear interpolation of the data. The test material data on nominal and measured concentrations of DME in solution is provided in the Table below (Table 4.2(a)). All measured concentrations exceeded nominal values for all exposure levels. Environmental and water quality parameters were within acceptable ranges e.g., pH (6.8 – 8.5) and D.O.= >4.0 ppm.

Table 4.2(a)

Test Solution (mg/L)	Measured concentration of DBE (DME) (mg/L)
300	335.6
250	268.3
200	198.9
150	178.6
100	04.5
0	<5.0

Reliability: High (Scientifically defensible or guideline method, measured concentrations, GLP).

Reference: Monsanto. (1992). Determination of acute toxicity of mixed dimethyl esters (DME) to *Daphnia magna*. MO-92-9961.

Type: 48-h EC₅₀

Species: *Daphnia magna* (Water Flea).

Value: >112 ppm (v/v) and <150 ppm (v/v)

Method: *Daphnia* were exposed to DBE under static conditions in replicate glass chambers with 250 ml volume for 48 hours. Exposures were monitored for temperature, pH,

dissolved oxygen and other water quality parameters. The system was maintained as 16 hrs. light/ 8 hrs. dark photoperiod without aeration. Exposure levels were reported as nominal.

GLP: No
Reliability: Moderate (Scientifically defensible or guideline method, nominal concentrations, non GLP).
Reference: Dupont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 308-82.

4.3. Acute Toxicity to Aquatic Plants

Type: No Data
Species: No Data
Value: No Data
Method: No Data
GLP: No Data
Reliability: No Data
Reference: No Data

5. Mammalian Toxicity

5.1. Acute Toxicity

Type: Acute Oral LD50
Guideline: EPA (40 CFR) 798.1175
Species/strain: Rats/Crl:CD®
Sex: Male
Value: LD50 (rats) = 8191 mg/kg
Method: No Data
GLP: No Data
Reliability: Moderate (Scientifically defensible or guideline method, non-GLP)
Reference: Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 646-81.

Type: Oral LD₅₀
Guideline: EPA (40 CFR) 798.1175
Species/Strain: Rats/Crl:CD®(CD)BR
Value: > 500 mg/kg b. wt. and < 5000 mg/kg b. wt.
Method: DBE was administered a single dose via oral gavage at two dose levels (500 and 5000 mg/kg b.wt.) with a 14 day observation period. Test material was administered as received. 5 females and 5 males were used for each dose level. Necropsies were

	performed on animals that died and that survived to 14 days post-administration.
GLP:	Yes
Test Substance:	99.5% total dibasic esters (DBE, DME) (12% DMA, 62% DMG, and 26% DMS)
Results:	At 500 and 5,000 mg/kg b. wt. mortality rates were 0/10 and 8/10, respectively. The mortalities occurred in the first two days. In the 500 mg/kg treatment level the only abnormality was decreased food consumption and fecal volume for one of ten animals on days 2 and 3. In the 5,000 mg/kg treatment group observations included: yellow-cervical staining; decreased activity and lethargy, hunched appearance and labored breathing. Surviving animals showed no abnormalities and necropsies of dead animals revealed discoloration in the lungs (edema in one individual). Other observations in single animals were reddened pancreas and yellow fluid in the stomach.
Reliability:	High (Scientifically defensible or guideline method, GLP)
Reference:	Bio/dynamics Inc. (1992). Acute Oral Toxicity in Rats (DME, Dimethyl Ester). Submitted to Monsanto Company Reference Numbers: 92-6316, BD-92-242, October 22, 1992.
Remarks:	This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Oral Toxicity:

ALD² (rat) = 11,000 mg/kg (Dupont Co, 1965).

ALD (rat) = 17,000 mg/kg (Dupont Co, 1978).

Dupont Co. (1965). Unpublished Data, Haskell Laboratory Report No. 88-65.

Dupont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 219-78.

Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 646-81.

DBE is not a Class B poison under Dept. of Trans. Regulations

² Approximate Lethal Dose

Dupont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 686-77.

A method was developed for measuring the effects of toxicants on visual function. The method employed visual evoked potentials (VEPs) elicited with patterned and flashed visual stimuli as measures of the effects of toxicants on visual function. DBE when administered orally by gavage at doses of 20, 200, or 2000 mg/kg failed to alter the pattern of VEPs of rats. A similar response was seen in rats administered DBE by inhalation and by nasal drops with and without a carboxylesterase inhibitor.

Boyes, W.K., and H.K. Hudnell (1993). U.S. EPA Report dated 12-21-93 (C-2592).

Type:	Dermal LD50
Guideline:	EPA (40 CFR) 798.1100
Species/Strain	New Zealand White Rabbits
Value:	> 5000 mg/kg b. wt.
Method:	DBE was administered a single dose applied directly to the skin over a 12 x 14 cm area approximating 10% of the body surface. Contact with excess material was maintained for 24 hours and animals were observed for 14 days after initiation of dose. A total of 5 females and 5 males were used for this study. Test material was administered as received. Study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.
GLP:	Yes
Test Substance:	99.5% total esters (DME) (12% DMA, 62% DMG, and 26% DMS)
Results:	All animals survived after dermal treatment at 5,000 mg/kg b. wt. Animals gained weight during 7 days post-treatment, but all animals lost weight or remained stable from day 7 to 14 post-treatment. All animals were free of systemic toxicity throughout the study and no abnormalities were observed during post-mortem macroscopic observation. However, one animal exhibited fecal staining (days 2 and 3), and one animal exhibited reddening at the site of application.
Reliability:	High (Scientifically defensible or guideline method, GLP)

Reference: Bio/dynamics Inc. (1992). Acute Dermal Toxicity in Rabbits (DME, Dimethyl Ester). Submitted to Monsanto Company Reference Numbers: 92-6317, BD-92-242, October 22, 1992.

Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Dermal Toxicity:

LD50 (rabbits) = > 2250 mg/kg. b.wt.

Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 634-81.

LD50 (rabbits) = >5000 mg/kg b.wt. IUCLID (2000)

DBE did not produce deaths in rabbits at doses up to 3400 mg/kg. No toxic or pathologic signs were observed.

Dupont Co. (1965). Unpublished Data, Haskell Laboratory Report No. 88-65.

Type:	Inhalation 4-hour LC50
Species/strain:	Rats/Crl:CD [®] (CD)BR
Value:	4-hour LC50 (rat) = > 11 mg/L.
Method:	Groups of five male and five female rats were exposed nose-only for a single 4-hour period to aerosol/vapor mixtures of DBE in air. Possible ocular effects were evaluated in one group of four male rats exposed whole-body. Indirect ophthalmoscopy was used at 30 minutes and at 1 day following exposure. Animals were observed for clinical signs during a 14-d observation period.
GLP	No
Test Substance:	99% total dibasic esters (DBE, DME) (17% DMA, 66% DMG, and 16.5% DMS)
Results:	No deaths occurred following exposure to DBE at concentrations as high as 11 mg/L, the highest concentration tested. Clinical signs of toxicity noted either during or immediately after exposure to DME concentrations of 5.6 mg/L or greater included red ocular or nasal discharges, lethargy, labored breathing, or hunched posture. One day after exposure, rats exhibited slight to severe weight loss and

some rats had red nasal, ocular, or oral discharges, wet, yellow-stained perineum, and hunched posture. With the exception of sporadic, slight to moderate weight losses in some rats during the first or second weeks of the recovery period, all clinical signs were transient and had resolved by study day four. Ophthalmologic examinations revealed only mild chemosis (swelling or edema) in the bulbar conjunctiva of the rats from the 5.6 mg/L of DBE .

Reliability: Moderate (Scientifically defensible or guideline method, non-GLP).
 Reference: Dupont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 453-90.
 Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Inhalation Studies:

4-hour ALC³ (rat) = 4.1 mg/L (Dupont Co, 1977)

4-hour ALC (rat) = 4.5 mg/L (Dupont Co 1978)

4-hour ALC (rat) = 6.1 mg/L (Dupont Co, 1965)

In these studies, clinical signs of toxicity during exposure included labored respiration, eye irritation, and a red-tinged nasal discharge. The rats recovered within two days after exposure.

Dupont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 535-77.

Dupont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 471-78.

Dupont Co. (1965). Unpublished Data, Haskell Laboratory Report No. 88-65.

1-hour LC50 (rat) = > 10.7 mg/L. No deaths occurred in a group of 10 rats exposed for one hour to 10.7 mg/L of an aerosol of DBE. The mass medium diameter of the aerosol was 5.4 mm. No signs of toxicity were observed.

Dupont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 436-80.

Dupont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 453-90.

Eye Irritation and Ocular effects:

³ Approximate Lethal Concentration

Mild Ocular irritation was observed one hour post-exposure following a four-hour inhalation exposure to 15 or 60 ppm of DBE. No Changes in Anterior Chamber Depth (ADC) were observed.

Dupont Co. (1992). Unpublished Data, Haskell Laboratory Report No. 362-92.

Inhalation exposure at 60 ppm for up to one hour failed to alter visual evoked potentials (VEPs) in rats.

Boyes, W.K., and H.K. Hudnell (1993). U.S. EPA Report dated 12-21-93 (C-2592).

Olfactory Effects:

Lee, K.P. et al. (1992a). The Toxicologist, 21(1):398 (Abstract 1571).

Lee, K.P. et al. (1992b). Toxicol. Pathol., 20(3, Part 1):376-393.

Morris, J.B. et al. (1991) Toxicol. Appl. Pharmacol., 108(3):538-546.

Type:	Primary Dermal Irritation
Guideline:	EPA (40 CFR) 798.4470
Species/Strain	New Zealand White Rabbits
Value:	Average Dermal Irritation Score (ADIS) was 0.0
Method:	DME (DBE) was administered to six animals (3 female/3 male) as a single dose applied directly to two 1 x 1 inch areas of skin on the back and held in place with semi-occlusive dressings for 4 hours. Animals were observed for subsequent 3 days and treated areas were observed at 30 minutes and 24, 48, and 72 hours. Test material was administered as received.
GLP:	Yes
Test Substance:	99.5% total dibasic esters (DBE, DME) (12% DMA, 62% DMG, and 26% DMS)
Results:	The ADIS for DME is 0.0. No irritation was observed with the exception of "barely perceptible" erythema in one of two treated areas in one animal at 0.5 hours after removal. This material would probably not be considered to produce dermal irritation as defined in EPA Guidelines.
Reliability:	High (Scientifically defensible or guideline method, GLP).
Reference:	Bio/dynamics Inc. (1992). Primary Dermal Irritation Study in Rabbits (DME, Dimethyl Ester). Submitted to Monsanto Company

Reference Numbers: 92-6318, BD-92-242,
October 22, 1992.

Remarks: This study was designed as one in a series of
four acute studies conducted with each of
four different dibasic ester test materials:
DBE (mixture), DMA, DMG, and DMS.

Additional References for Dermal Irritation:

Pure DBE (100%) and a 10% emulsion in water were tested on the skin of male and female guinea pigs. During the primary irritation phase, no dermal irritation was observed. DBE did not produce delayed hypersensitivity or allergic reactions (Dupont Co, 1989a). DBE was evaluated for acute skin irritation in three male and three female rabbits. One rabbit exhibited mild erythema by 24 hours following treatment. Two rabbits exhibited moderate erythema within 48 hours of treatment and one of these had slight edema. By 72 hours, one rabbit exhibited severe erythema with fissuring of the skin and slight edema. No moderate to mild erythema and no edema were observed in the other five rabbits at 72 hours after treatment (Dupont Co, 1989b).

Dupont Co. (1989a). Unpublished Data, Haskell Laboratory Report No. 71-89.

Dupont Co. (1989b). Unpublished Data, Haskell Laboratory Report No. 77-89.

To determine if dermal exposure to DBE could cause eye irritation or other ocular effects, a group of four rabbits was dermally administered 50 ml (20 mg/kg) of DBE. No DBE-related abnormalities were observed in the cornea, lens, or retina by indirect ophthalmoscopy. A slight, but not statistically significant, increase in the anterior chamber depth (ACD) in the eye (3-6%) was observed. No changes in corneal thickness were observed. In an attempt to reproduce this effect on ACD, two other groups of four rabbits were dermally administered 200 ml (80 mg/kg) of DBE. No changes in ACD were noted in one study, while a minimal (2.3%) increase was noted in the other (Dupont Co, 1992).

Dupont Co. (1992). Unpublished Data, Haskell Laboratory Report No. 362-92.

IUCLID (2000)

DBE is not a Class B poison under Dept. of Trans. Regulations when tested by the skin absorption route.

Dupont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 834-77.

Dupont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 127-78.

Type:	Eye Irritation
Guideline:	EPA (40 CFR) 798.4500
Species/Strain	New Zealand White Rabbits
Value:	DME (DBE) produced mild to moderate, transient ocular irritation.
Method:	A single ocular administration of DBE (0.1 ml) was applied to 3 male and 3 female adult rabbits followed by observations at 1, 24, 48, 72 hours and 7 and 10 days. The observation period continued up to 10 days or until no signs of irritation were present. The cornea, iris and conjunctivae were observed and lesions were graded. Test material was administered as received.
GLP:	Yes
Test Substance:	99.5% total esters (DBE) (12% DMA, 62% DMG, and 26% DMS)
Results:	DBE produced mild to moderate, transient ocular irritation. This material would be considered to produce eye irritation as defined in the EPA Guidelines. All six animals exhibited slight to moderate irritation of the conjunctivae (redness, chemosis, and discharge) and three exhibited iridial changes. Three animals exhibited slight changes in opacity or ulceration of the cornea. One animal had no positive scores throughout the study, four of six animals were clear of irritation within 48 hours, and the remaining animal was clear by 7 days.
Reliability:	High (Scientifically defensible or guideline method, GLP).
Reference:	Bio/dynamics Inc. (1992). Primary Eye Irritation Study in Rabbits (DME, Dimethyl Ester). Submitted to Monsanto Company Reference Numbers: 92-6319, BD-92-242, October 22, 1992.
Remarks:	This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Eye Irritation:

Instillation of 0.1 ml of DBE into the eyes of rabbits produced minimal conjunctival irritation. Prompt washing reduced the degree of irritation. All treated eyes had returned to normal 24 hours after treatment.

Dupont Co. (1974a). Unpublished Data, Haskell Laboratory Report No. 108-74.

Instillation of 0.1 ml of a 1% aqueous DBE solution into a rabbit eye produced a localized area of slight corneal opacity and transient conjunctival irritation. The treated eyes were normal three days after.

Dupont Co. (1974b). Unpublished Data, Haskell Laboratory Report No. 483-74.

Instillation of 0.1 ml of DBE into the eyes of rabbits produced mild to slight clouding of the cornea, which became slight in eight days. Immediate washing decreased the clouding.

Dupont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 858-80.

A crude sample of DBE containing 21% methanol produced severe corneal opacity, moderate iritis, and severe conjunctival irritation in rabbits. The injury did not appear to be reversible and washing with tap water or a 25% propylene glycol solution did not significantly reduce the injury. This material is not typical of the DBE used as an industrial solvent.

Dupont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 120-78.

DBE (10 ml) was instilled into the rabbit eye. Mild corneal opacity was observed in 2/4 of the rabbits. This opacity cleared in one rabbit by two days after treatment. No changes in the anterior chamber depth were found.

Dupont Co. (1992). Unpublished Data, Haskell Laboratory Report No. 362-92.

Imsol R (an ICI equivalent of DBE) was instilled into the eyes of groups of three rabbits at 0.01, 0.03, and 0.1-ml. Moderate initial pain was noted in all the rabbits administered 0.01 ml and evidence of corneal anesthesia was seen in two rabbits, which persisted for up to 1 hour after treatment. All the rabbits showed conjunctival effects, ranging from slight redness to moderate redness and ocular discharge, persisting up to eight days after treatment. Corneal or iridal effects were not seen in 2/3 rabbits, but the third showed

slight to moderate corneal opacity between days 3 and 8, reaching a maximum on days 4-5. Significant corneal swelling (44%) was observed in this rabbit with the maximum response on day one. Corneal swelling and corneal surface irregularity persisted in this rabbit up to day eight. Moderate initial pain and corneal anesthesia was again evident in the rabbits administered 0.03 ml, which was of a similar duration as seen in the 0.01 ml group. Conjunctival effects of slight to moderate redness, slight chemosis, and slight to severe discharge were observed, with the highest response on day three and full regression by day seven. Evidence of corneal opacity was apparent until day seven, with slight iritis from day two to day six. Significant corneal swelling (25%) was observed on day two, again accompanying signs of corneal surface irregularity. In the group administered 0.1 ml, a similar pattern of pain reaction, and corneal anesthesia was noted. Conjunctival effects (slight to moderate redness, slight chemosis, and slight to moderate discharge) persisted up to day 10. Slight to moderate corneal opacity covering up to the entire area of the cornea occurred up to day six, with the maximum response on days 3-4. Significant corneal swelling (53%) was seen on day two. All indications of ocular irritancy had regressed by day 10.

ICI, Ltd. (1991). Central Toxicology Laboratory, Report CTL/T/2750 (J-8948).

5.2. Repeated Dose Toxicity

Type:	Oral Gavage
Species/Strain	Sprague-Daley (CD) rats
Sex/Number	10/sex/dose
Exposure	
Period:	One month
Frequency	
of Treatment:	Daily, seven days per week.
Levels:	0, 100, 300, and 1,000 mg/kg/day
Method:	Groups of 10 animals/sex/dose were dosed daily for one month via oral gavage at 0, 100, 300, and 1,000 mg/kg/day. The test material DBE (DME) (mixture of 12% DMA, 62% DMG, and 26% DMS; 99.5% total esters) was administered in corn oil with corn oil control. Concentrations were verified using gas-liquid chromatography. Water and food were available <i>ad libidum</i> . In-life observations included: mortality and other signs of toxicity, body weight, and food consumption. Clinical observations included: body weights, food consumption, and clinopathological measurements. All

	animals were necropsied at test termination and tissues were retained.
GLP:	Yes
Test Substance:	DBE (DME) (99.5% total dibasic esters) (mixture of 12% DMA, 62% DMG, and 26% DMS)
Results:	Analytical confirmation of dose solutions showed from 0.0 to 8.0% below target concentrations. Relative to control animals there were no significant differences observed in treated group mean body weights or cumulative body weight gains, no unscheduled mortalities, no treatment related results from ophthalmoscopic examination, no treatment-related changes in food consumption, clinical signs, or gross or microscopic pathology. The one exception is a small decrease in urine pH in males (non significant) and female at 1,000 mg/kg/day dose level. The authors stated this could have been due to excretion of test material, but there was no evidence on any direct or indirect effect on the urinary system. The 1,000 mg/kg/day dose level was considered an NOAEL.
Reliability:	High because scientifically defensible or guideline, and GLP
Reference:	Monsanto. (1992). One-Month Gavage Study of DME (Dimethyl Esters) in Sprague Dawley Rats. Report # MSL-12509
Type:	90-d Inhalation Study
Species:	Rats/Crl:CD [®] (CD)BR
Value:	No No-Effect Level
Method:	Groups of 10 male and 10 female rats were exposed six hours a day, five days a week, for approximately 14 weeks to DBE vapor concentrations of 160 or 400 mg/m ³ or to 1000 mg/m ³ of a DBE aerosol-vapor mixture.
GLP:	Yes
Test Substance:	99.5% total esters (DBE) (16.5% DMA, 66.6% DMG, 16.9%).
Results:	Histopathological evaluation of nasal tissues showed degeneration of the olfactory epithelium in all DBE-exposed groups. The effect was minimal in the 160 mg/m ³ group and of mild to moderate severity in the 400 and 1000 mg/m ³ groups. Other than the nasal lesions, histopathological examination showed no deleterious effects in DBE-

exposed rats at any tested concentration. Other effects of DBE exposure included dose-dependent decreased liver/body weight ratios in male and female rats in the 400 and 1000 mg/m³ groups, and slightly increased lung/body weight ratios and slightly depressed body weights in male and female rats in the 1000 mg/m³ group. A no-effect exposure concentration was not demonstrated.

Reliability: High (Scientifically defensible or guideline method, GLP)

References: Dupont Co. (1986). Unpublished Data, Haskell Laboratory Report No. 194-86. Kelly, D.P. et al. (1986). The Toxicologist, 6(1):136 (Abstract 551).

Type: **90-day Inhalation**

Species/Strain: Rats/ Crl:CD®(SD)IGS BR

Sex/Number: 40 male and 40 female/treatment level

Route of Administration: inhalation

Exposure Period: 13 wk

Frequency Of treatment: 6 hours/day, 5 days/wk

Exposure levels: 20,76, or 390 mg/m³ of DBE.

Type: **90-d Inhalation Study**

Species: Rats/Crl:CD®(CD)BR

Value: NOEC = 20 mg/m³ in males rats relative to changes in the olfactory mucosa.

Method: Ten rats per group were sacrificed after approximately seven weeks of exposure, and 20 rats per group were sacrificed after approximately 13 weeks of exposure. The remaining 10 rats in each group were sacrificed after a recovery period of six weeks. Gross necropsy and nasal histopathological examinations were conducted on all control and DBE-exposed rats.

GLP: Yes

Test Substance: 99.5% total esters (DBE) (16.5% DMA, 66.6% DMG, 16.9% DMS)

Results: Degeneration of the olfactory epithelium was observed in male and female rats that were exposed to 76 or 390 mg/m³ of DBE for seven weeks. After 13 weeks of exposure, there was degeneration of the olfactory epithelium in female rats in all

exposure groups and in male rats exposed to 76 or 390 mg/m³. At the end of the 6-week recovery period, DBE exposure-related effects were still visible in affected groups, and histological changes were compatible with repair of the olfactory mucosa. During the exposure period, clinical signs in DBE-exposed rats were similar to those of the controls. However, female rats exposed to 390 mg/m³ of DBE had slightly depressed rates of weight gain compared to controls during the exposure period. During the recovery period the weight-gain rate of these rats was similar to those of the controls. At the end of the exposure period, absolute liver weights in female rats exposed to 390 mg/m³ were decreased compared to those of the controls. This effect was not present in rats at the end of the recovery period. In the absence of corroborative histopathological findings, the biological significance of this effect is not known. A slight decrease in serum sodium concentration was observed in male and female rats exposed to 76 or 390 mg/m³ of DBE for 13 weeks. After the six-week recovery period, sodium concentrations in the 390 mg/m³ group remained slightly low, but the sodium concentrations in the other affected groups were no longer different from those of the control group. The slightly decreased sodium concentrations were considered to be of minimal biological significance. A no-effect exposure concentration of 20 mg/m³ was demonstrated in male rats for the olfactory epithelial degeneration observed. A no-effect concentration was not demonstrated in female rats.

- Reliability: High (Scientifically defensible or Guideline, GLP)
- References: Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 312-87.
 Keenan, C.M. et al. (1988). The Toxicologist, 9(1):284 (Abstract 23).
 Keenan, C.M. et al. (1990). Fundam. Appl. Toxicol., 15(2):381-393.

Additional References for Repeated Dose Inhalation:

Groups of 10 rats were exposed six hours a day, five days a week, for two weeks to 0.1, 0.3, or 0.9 mg/L of DBE. The exposed rats

were indistinguishable from controls in terms of growth and histopathological examination.

Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 244-81.

Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 815-81.

Morris, J.B. et al. (1991) Toxicol. Appl. Pharmacol., 108(3):538-546.

5.3. Developmental Toxicity

Type:	Teratogenicity
Species/Strain:	Rats/Crl:CD [®] (SD)BR
Sex/Number:	24 female (pregnant)
Route of Administration:	Inhalation
Exposure Period:	Days 7-16 of gestation
Frequency of Treatment:	Six hours/day.
Value:	NOECs for the dam and fetus are 160 and 1000 mg/m ³ , respectively.
Method:	Groups of 24 pregnant rats were exposed six hours a day, on days 7 -16 of gestation to 160, 400, or 1000 mg/m ³ of DBE.
GLP:	Yes
Test Substance:	99.5% total esters (DBE) (16.8% DMA, 65.1% DMG, 17.8% DMS)
Results:	Maternal body weight gains were significantly reduced during the period of administration (days 7 -16) of DBE at 400 and 1000 mg/m ³ ; the reduction on days 9-11 was also significant. Maternal feed consumption was significantly reduced at the same exposure levels during the first six days of DBE exposure (days 7-9, 9-11, and 11-13). Clinical observations related to treatment (increased staining of the periocular, perinasal, and head fur, and wet fur) were significant only at 1000 mg/m ³ and were not severe. No other evidence of maternal toxicity was seen. No effects on fetal survival, fetal weight, litter size, or nidations were seen. The incidence of fetal malformations and variations showed no exposure-related changes. Since maternal toxicity was demonstrated at 400 mg/m ³ and fetal toxicity was absent at the highest

	exposure level, 1000 mg/m ³ , the NOEL for the dam and fetus are 160 and 1000 mg/m ³ , respectively (Dupont Co, 1987e; Kelly et al., 1986).
Reliability:	High (Scientifically defensible or guideline, GLP)
References:	Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 562-87. Kelly, D.P. et al. (1986). <u>The Toxicologist</u> , 6(1):136 (Abstract 551).

5.4. Reproductive Toxicity

Type:	Repeated Inhalation (14 week)
Species/Strain	Rats/Crl:CD [®] (SD)BR
Sex/Number	20 pairs (male and female)
Exposure Period:	14 weeks
Frequency of Treatment:	Daily, five days per week.
Method:	Groups of 20 male and 20 female rats were mated after exposure for six hours a day, five days a week, for about 14 weeks to DBE vapor concentrations of 160 or 400 mg/m ³ , or to 1000 mg/m ³ of a DBE aerosol/vapor mixture. DBE exposure continued during breeding (15 days), gestation (21 days), and lactation (21 days) periods. DBE exposures were discontinued for the dams after the 19th gestation day and begun again on day four postpartum. Offspring were not subjected to DBE exposure.
GLP:	Yes
Test Substance:	99.5% total esters (DBE) (16.8% DMA, 65.1% DMG, 17.8% DMS).
Results:	The only DBE exposure-related effect was depressed pup weights in the 1000 mg/m ³ exposure group from days 1-21 postpartum. No exposure-related differences were observed between control and test groups in the following reproduction parameters: male and female fertility indices, born-alive index, viability index, gestation index, and lactation index. A gross pathological examination of 21-day old rats whose parents had been exposed to DBE showed no exposure-related effects.
Reliability:	High (Scientifically defensible or guideline, GLP)

References: Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 76-87.
Kelly, D.P. et al. (1986). The Toxicologist, 6(1):136 (Abstract 551).

Additional References for Repeated Dose Inhalation: Not found

5.5. Genetic Toxicity

Type: *In vitro* Bacterial Reverse Mutation Test
Tester Strains: *Salmonella typhimurium* strains TA98 and TA100, and TM677
Exogenous Metabolic Activation: Rat liver (S-9) fraction/female rat olfactory mucosa activation
Exposure Concentrations: Maximum of 8.53 mg DBE/treatment (1.7 mg/ml) in Reverse Suspension Assay// maximum of 100 µg DBE/treatment (0.9 mg/ml) Microforward Suspension Assay
Methods: DBE was tested for mutagenic activity in *Salmonella typhimurium* strains TA98 and TA100 in the presence and absence of a rat-liver activation system using a suspension assay, and in a microforward mutation assay in strain TM677 in the presence of female rat olfactory mucosa activation.
GLP: Yes
Test Substance: 99.5% total esters (DBE) (15.9% DMA, 68.9% DMG, 14.9% DMS).
Results: Negative.
Reliability: High (Scientifically defensible or guideline, GLP)
Reference: Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 584-87.
Vlachos, D.A. et al. (1988). Environ. Mol. Mutagen., 11 (Suppl. 11):109.

Additional References for *in vitro* Bacterial Reverse Mutation Test:

DBE was not mutagenic in the Ames *Salmonella* test, either in the presence or absence of an S-9 activation system.

Dupont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 396-77.

Dupont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 462-80.

Type:	<i>In vitro</i> Chromosome Aberration Study
Cell Type:	Human lymphocytes
Exposure	
Concentrations:	≥ 0.3% v/v (3.3 mg/ml, based on approximate average molecular weight of components).
Methods:	DBE was evaluated for <i>in vitro</i> clastogenic (chromosome damaging) activity in human lymphocytes with and without metabolic S-9 activation. Additional work was undertaken to elucidate the accuracy of these findings. Based on these results, it was suspected that the positive <i>in vitro</i> findings with DBE resulted from an acidic culture environment produced by this chemical under activated conditions. Therefore, experiments were undertaken to more thoroughly evaluate the pH changes induced by DBE under activated conditions and the apparent sex- specific response.
GLP:	Yes
Test material:	99.5% total esters (DBE)
Results:	As demonstrated by cell-cycle delay and/or reduced mitotic index, cytotoxicity was observed at DBE concentrations, ≥ 0.3% v/v (3.3 mg/ml, based on approximate average molecular weight of components). Chromosome aberration analyses following non-activated treatments showed no statistically significant increases in the percent abnormal cells in cultures treated with DBE. Under activated conditions, however, statistically significant increases in structural chromosome aberrations were reproducibly observed at DBE concentrations, ≥ 0.3% v/v. These aberrations were predominant in cells from female (but not male) donors. Under the conditions of this assay, DBE is clastogenic. In the above study, measurements of pH were made with culture medium pooled from male and female cultures following the completion of the three-hour treatment period. In the presence of S9, acidic conditions were observed at concentrations of 3.3 and 4.4 mg/ml. In contrast, the pH of the culture medium from non-activated 4.4 and 6.6 mg/ml treatments were similar to control.

Reliability:	High (Scientifically defensible or guideline method, GLP).
References:	Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 531-87. Vlachos, D.A. et al. (1988). Environ. Mol. Mutagen., 11 (Suppl. 11):109.
Remarks:	Under activated conditions only, DBE causes reductions in the pH of culture medium. This was demonstrated in two independent assessments, where concentrations of DBE similar to those inducing positive results were evaluated. The drop in pH occurs quickly following the addition of DBE, and the resulting pH is in the range that has been reported to induce clastogenic effects with activation. The induction of chromosome aberrations by DBE was quite variable based on the number of positive and negative results that have been produced with activation. The variability is believed to be attributable, in part, to the severe toxicity that this chemical induces.

Additional References for *in vitro* Chromosome Aberration Study:

None found

Type:	<i>In vivo</i> Rat Erythrocyte Micronucleus Test
Guideline:	US EPA (1998). Health Effects Test Guidelines. OPPTS 870.5395
Species/strain:	Mice/Crl:CD®(ICR)BR
Sex/Number:	4 male and 4 female mice per treatment level
Route of Administration:	Inhalation (nose only)
Exposure Concentrations:	5.5, 11, or 19 mg DBE/L
Exposure Duration:	Six hours/one exposure
Method:	DBE was tested for its ability to induce micronuclei in the bone-marrow polychromatic erythrocytes of male and female mice. The mice were exposed nose-only to atmospheres of 5.5, 11, or 19 mg/L of DBE aerosol in air for 6 hours. Bone-marrow smears were prepared approximately 24, 48, and 72 hours after the beginning of exposure, and 1000

	polychromatic erythrocytes per mouse were scored for the presence of micronuclei.
GLP:	Yes
Test Substance:	99.5% total esters (DBE)
Results:	Significant depression in the ratio of young, polychromatic erythrocytes to mature, normochromatic erythrocytes was detected in the 11 and 19 mg/L treated females at the 24-hour sampling as compared to their concurrent negative controls. No statistically significant increases in the frequency of micronucleated cells were seen in the DBE-treated mice at any sampling time. Under the conditions of this assay, DBE does not induce micronuclei.
Reliability:	High (Scientifically defensible or guideline, GLP)
References:	Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 498-87. Vlachos, D.A. et al. (1988). Environ. Mol. Mutagen., 11 (Suppl. 11):109.

6.0 Other Information

6.1 Biochemical/Metabolism Studies

Hepatic mitochondria were used as a model for nasal tissue. DBEs were found to inhibit mitochondrial ATP synthesis 11 to 27% at 100 μ M. The order of potency was DMA > DMG > DMS and paralleled the V_{\max}/K_m values for the hydrolysis of the DBEs to their monomethyl esters. Pre-treatment of the rats with 100 mg/kg of bis-nitrophenyl phosphate for three days decreased the rate of hydrolysis of the DBEs approximately 50% and protected the mitochondria from DBE-induced inhibition of ATP synthesis. These results support the hypothesis that DBE-induced cytotoxicity results from esterase-mediated hydrolysis to acid metabolites and interference with intermediary metabolism (Bogdanffy and Londergan, 1989).

In the study cited above, DBE cytotoxicity was shown to be due to esterase-mediated activation. In this present study, the putative toxic monomethyl and diacid metabolites were evaluated in an *in vitro* nasal explant system. Monomethyl adipate (MMA), glutarate (MMG), and succinate (MMS) induced increases in nasal explant acid phosphatase release, a biochemical index of their cytotoxicity. Metabolism of MMA and MMG to their diacids paralleled cytotoxicity. MMS metabolism was not quantifiable. Pretreatment of rats with a carboxylesterase inhibitor reduced cytotoxicity and metabolism of MMA and MMG, but not cytotoxicity of MMS. It is concluded that both monomethyl ester and diacid metabolites of DBE are cytotoxic. The contribution of each to cytotoxicity *in vivo* may depend on their rate of formation during exposure (Bogdanffy et al., 1991a; Trela and Bogdanffy, 1991b).

The kinetic parameters V_{\max} , K_m , K_{si} , and V/K were measured for the hydrolysis of the dibasic esters in the target nasal tissue, olfactory mucosa, and non-target tissue, respiratory mucosa. It was determined under the conditions of these experiments, diacid metabolites were not formed. Esterase activity was inhibited by pretreatment with bis-nitrophenyl phosphate. V_{\max} values for the three dibasic esters were 5- to 13-fold greater in olfactory mucosa than respiratory mucosa for male and female rats. V/K values were 4- to 11-fold greater in olfactory mucosa than respiratory mucosa for male and female rats. V/K was similar between male and female olfactory mucosa when DMG was used as the substrate. With DMS or DMA as the substrate, V/K for female olfactory tissue was 0.5- or 2-fold that of males, respectively. Differences in V/K were mainly due to decreases in K_m associated with increasing carbon chain length. Substrate inhibition was observed at DBE concentrations greater than approximately 25 mM, which are unlikely to be achieved *in vivo*. These results lend further support to the hypothesis that organic acid accumulation in the target tissue, olfactory mucosa, plays a significant role in the pathogenesis of DBE-induced nasal lesions (Bogdanffy et al., 1991b).

Since female rats appear to be more sensitive to DBE-induced olfactory toxicity than males, it was of interest to measure the rate of hydrolysis of DBEs in male and female nasal mucosa homogenates and compare these values to those derived from human nasal tissue obtained at autopsy. For both male and female rats, V_{\max}/K_m values followed the order DMA > DMG > DMS paralleling carbon chain length. The V_{\max}/K_m values for female olfactory mucosa using DMA or DMS as substrates were two times or one-half the values for male olfactory mucosa, respectively. Hydrolysis of DBEs was detectable in only three of six human samples. Activity values that were measurable were two or three orders of magnitude lower than that of rat respiratory or olfactory mucosa, respectively. These data suggest the rate of conversion of DBEs to acid metabolites in nasal tissue is less significant in humans than in rats, and that the rat may be more sensitive than man to the effects of DBEs on nasal mucosa (Kee et al., 1989).

The enzymatic esterase activity of carboxylesterases is integral to the nasal toxicity of many esters, including DMG, DMS, and DMA. Inhalation of these esters specifically damages the olfactory mucosa of rodents. In this study, the localization differential distribution of a 59 KD carboxylesterase was demonstrated in the nasal tissues of the rat by immunochemistry. Rabbit antiserum against the 59 KD rat liver microsomal carboxylesterase bound most prominently to the olfactory mucosa when applied to decalcified, paraffin-embedded sections of rat nasal turbinates. Within the olfactory mucosa, anti-carboxylesterase did not bind to sensory neurons, the target cell for ester-initiated toxicity; these cells apparently lack carboxylesterase. Instead, the antibody was preferentially bound by cells of Bowman's glands and sustentacular epithelial cells that are immediately adjacent to the olfactory nerve cells. In contrast, non-olfactory tissues (respiratory mucosa and squamous epithelium) which are more resistant to the toxicity of esters, had less carboxylesterase content (Olson et al., 1993).

An *in vitro* system was utilized to determine if DBE toxicity is dependent on metabolic activation by carboxylesterase. Explants from the olfactory and respiratory regions of the rat nasal cavity were incubated in a medium containing 10-100 mM of the dimethyl esters of adipic-, glutaric-, and succinic acids. DBE caused a dose-related increase in nasal explant acid phosphatase release, a biochemical index of cytotoxicity. A parallel increase in carboxylesterase-mediated monomethyl ester (MME) formation was seen. In addition, MME concentrations and acid phosphatase release were generally higher in olfactory than respiratory tissues. DME-induced cytotoxicity and MME formation were markedly reduced in nasal tissue excised from rats treated with a carboxylesterase inhibitor, bisnitrophenyl phosphate (Trela and Bogdanffy, 1990; 1991a).

The kinetic constants were determined for carboxylesterase-mediated hydrolysis of DBEs and correlated with lesion formation. No diacid metabolites were found. V max values for the formation of MMS, MMG, and MMA were approximately 8- to 10-times larger in olfactory mucosa than in respiratory mucosa. V/K values for the formation of MMG and MMA were approximately 9- and 10-times larger in olfactory mucosa than respiratory mucosa. For the formation of MMS, V/K was approximately 2 times larger in respiratory mucosa than olfactory mucosa (Patterson et al., 1988).

To determine the biochemical mechanism for the toxic effect of DBE on rat nasal olfactory mucosa, an *in vitro* study was conducted with rat and human nasal tissue. This study demonstrated that the nasal tissue toxicity of DBE is related to enzymatic hydrolysis of DBE within the nasal cavity to form the corresponding monomethyl ester. Additionally, it was found that human nasal tissue hydrolyzes DBE at 1/100 to 1/1000 the rate of rat nasal tissue. For this reason, the nasal tissue of humans is likely to be at greatly reduced risk of DBE toxicity compared to rats (Bogdanffy and Frame, 1994).

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